



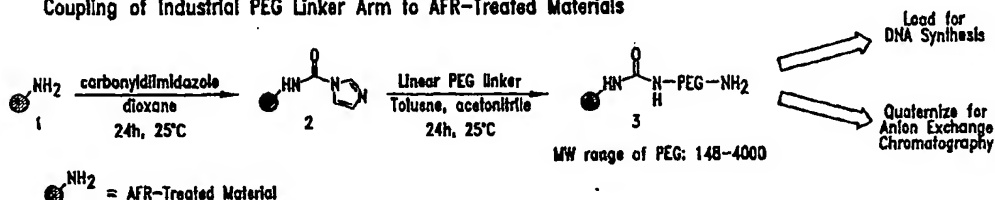
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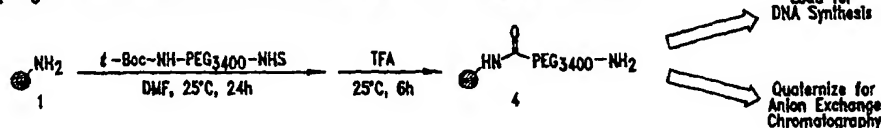
(54) Title: POROUS ARTICLES WITH SURFACE FUNCTIONALITY AND USES THEREOF

Modification of AFR-Treated materials with Linear PEGs for Use in DNA Synthesis and Anion Exchange Chromatography

Coupling of Industrial PEG Linker Arm to AFR-Treated Materials



Coupling of Well-Characterized PEG Linker Arm to AFR-Treated Materials



(57) Abstract

Porous organic articles having no surface functionality may be treated by remote plasma discharge to introduce oxygen or nitrogen atoms directly bonded to the surface of the article, including the interstitial surface. These oxygen and nitrogen atoms provide chemical handles through which a variety of useful moieties can be bonded to the article. Modified porous organic articles are useful in the solid phase synthesis of biomolecules when nucleosides, amino acids, or their derivatives are bonded directly or indirectly to the nitrogen or oxygen atoms. Alternatively, porous solid supports can be treated to introduce chromatographically active groups, such as an anion exchanger, a cation exchanger, an hydrophobic group, a hydrazide group, a reactive group of covalent bond formation through protein amino groups, a substituent group for reversed phase chromatography, and a substituent group for chiral chromatography. Moreover, surface-functionalized porous articles comprising an immobilized diagnostic agent can be used for diagnosing or monitoring patient conditions and diseases.

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POROUS ARTICLES WITH SURFACE FUNCTIONALITY AND USES THEREOF

TECHNICAL FIELD

The present invention relates generally to methods and devices
5 employing surface-functionalized porous articles. The invention is more particularly
related to the use of surface-functionalized porous articles in liquid chromatography,
biochemical synthesis, and for solid phase diagnostic and monitoring assays.

BACKGROUND OF THE INVENTION

Porous solid materials are now widely utilized in a variety of procedures
10 including chromatography, synthesis and purification of organic and biochemicals, and
assays to diagnose or monitor disease. For example, the synthesis of biochemicals,
such as oligonucleotides and polypeptides, can be performed using solid phase supports
in order to greatly reduce the time and expense arising from the inevitable purifications
needed between intermediate steps in a multi-step chemical synthesis. The practice of
15 solid phase synthesis is also particularly amenable to automation, thus allowing
significant cost-savings in terms of labor. These advantages have prompted a great deal
of research activity directed to improvements in solid phase synthesis, and one area of
keen interest is the development of superior solid supports.

In general, the efficiency of a process that uses a solid phase support
20 depends on the surface area of the solid phase material. Porous materials offer the
advantage of higher surface area per unit volume than the corresponding full density
solids. This characteristic permits vastly improved biochemical synthesis performance
per unit volume of solid material. The performances of solid phase diagnostic assays
and solid phase chromatographic techniques are also enhanced by the increased surface
25 area.

One popular support is made from glass or silica, often in the form of
beads. Glass beads have several desirable properties. They are inert to most (although
not all) chemical reactions and are not easily crushed. In addition, glass has many
surface hydroxyl groups that can be used as "chemical handles" to link other useful
30 moieties. Glass is fundamentally inexpensive and can be made highly porous, so that
the beads have a high surface area/volume ratio and increased efficiency and sensitivity.
However, glass beads are also rather brittle, and thus not very stable to mechanical
action. Small pieces of beads (commonly termed "fines") can chip away and clog
filtration equipment.

In an attempt to avoid the physical deficiencies of glass, organic and inorganic polymers have also been employed as support materials. Polymers such as such as polyethylene, polypropylene, polystyrene, poly(styrene/divinylbenzene), polyurethanes, polyimide thermosets, furan resins, polyesters and the like, possess the
5 necessary physical properties, including high strength, ready transformation into an almost limitless number of solid shapes, inertness to many chemical and physical conditions, and a low manufacturing cost. However, chemically reactive surface functional groups are not constitutive parts of such base polymers. Those polymers that are have reactive functional groups, such as polyvinyl alcohol, functionalized
10 poly(styrene divinylbenzene) or CPGTM glass materials, do not have all the properties (e.g., strength, moldability, economic manufacture, etc.) that are desired for chromatographic, biochemical synthetic, and diagnostic applications.

Attempts have been made to introduce hydrophilic surfaces and/or chemically reactive functional groups onto a basically inert, preformed bulk polymer
15 article. Using such an approach, a low-cost polymer can first be formed into a desired shape so as to optimize mechanical strength, fluid flow and other end item performance properties. In theory, the surfaces of the support may then be modified to become chemically reactive while the strength, fluid flow and other characteristics of the base polymer article remain unchanged. In practice, however, attempts at surface
20 modification have been inefficient or have resulted in altered support properties.

For example, solid and porous supports may be exposed to strong oxidizing chemicals (e.g., a high concentration of alkali, chromic acid with sulfuric acid, sulfuric acid with nitric acid, or ammonium hydrogen fluoride and nitric acid, etc.;
25 see, e.g., U.S. Patent No. 5,510,195 and Japanese Patent 61-2100). However, these chemical treatments cause serious damage to the supports, including loss of mechanical strength and an increase in pore size because the walls of the pores are etched away by the harsh chemicals. This approach is also physically difficult, in that a porous material must be completely wetted with the oxidizing chemical in order to obtain complete treatment, and then the oxidizing chemical must be completely removed by extensive
30 washing procedures. The oxidizing chemicals are known to produce extensive etching of the polymer surface simultaneously with introduction of surface functional groups.

In another approach, a hydrophilic chemical is deposited onto (or impregnated into) a solid support, which may be porous. For example, a surfactant having a hydrophobic moiety and a hydrophilic moiety may be contacted with the
35 support, such that the hydrophobic moiety "burrows" into the support, leaving the hydrophilic moiety exposed. This approach does render the region near the support

surface hydrophilic, at least temporarily. However, the surfactant molecules are not permanently affixed to the support, and thus are susceptible to leaching. *See, e.g.*, U.S. Patent Nos. 5,209,849, 4,851,121, and 4,794,002. Entrapment functionalization is a somewhat similar alternative approach in which terminally functionalized oligomers become entangled with the polymer chains comprising a solvent swollen bulk polymer phase. Entrapment functionalization produces a more permanent modification but contaminates the bulk polymer with the swelling solvent and produces a low degree of functionalization. *See, e.g.*, U.S. Patent No. 4,794,002, and Japanese Patent Application 59-501049. In addition, it is often difficult to obtain a uniform coverage of the coating or modifying material.

In a related approach, a hydrophilic polymer precursor molecule is deposited onto the surfaces of a support (which may be porous), and then subjected to conditions that cause crosslinking of the precursor to form a crosslinked polymer. In this way, the tendency of the hydrophilic moieties to leave the hydrophobic support is diminished. *See, e.g.*, U.S. Patent Nos. 5,209,849, 4,885,077, 4,851,121, 4,794,002, and 4,717,479. However, when UV/visible radiation or plasma discharge/plasma polymerization techniques are used to initiate the crosslinking reaction, it is difficult for the radiation to penetrate into the pores of a porous material, so that uniform coatings are not produced. In addition, the coating is typically not uniform across the surface of the support, and the pore volume and pore size distribution are altered by the non-uniformly distributed volume of the crosslinked polymer coating. Precursors or incomplete reaction products which do not cross link completely will leach away from the support, and may thus cause failure of a device or article containing the support.

Ionizing radiation or plasma discharge exposure suffer from similar limitations in performance. Ionizing radiation damages the bulk polymer, although penetration into porous solids is uniform. While ionizing radiation has been used to induce surface graft polymerization, the resultant supramolecular structure is distinct from that described herein, in that no extended interpenetrating surface network structure is produced and the advantages of the extended interpenetrating surface network structure are not obtained. Immersion in the plasma discharge environment results in severe etching and collateral damage of the exterior surfaces of a porous polymeric solid so that uniform surface treatment is not obtained and device performance is compromised.

Other treatments used for generating a hydrophilic surface are similarly deficient. Corona discharge treatments may temporarily impart hydrophilicity to the surface of a hydrophobic article, however the hydrophilicity is not permanent, and if the

hydrophobic article is porous, the corona treatment results in highly detrimental change to the pore structure (see U.S. Patent No. 4,851,121). Radiation treatments, such as UV, electron gun or gamma ray treatment, may be applied to a hydrophobic surface. However, these high-energy types of irradiation will penetrate into the interior of the bulk polymer article, and cause bond breakage and a deterioration in the mechanical properties of the treated article (see U.S. Patent No. 5,209,849).

In sum, the prior art describes many approaches to providing a hydrophilic or reactive surface on a hydrophobic or inert polymeric article, including porous articles. None of these approaches, however, are entirely satisfactory in meeting the need for a solid support that can be as conveniently used in both research-scale and production scale solid phase synthesis, solid phase chromatography, and solid phase medical assays. The present invention achieves these and related goals, as described more fully herein.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides porous compositions and methods of using such compositions for chromatographic separations, solid phase biochemical synthesis, and diagnostic assays. In one aspect, the invention provides a porous article having an external surface, a bulk matrix and pores extending from the external surface into the bulk matrix, wherein the pores define a pore surface, wherein the bulk matrix is formed, at least in part, of an organic polymer comprising carbon and hydrogen atoms, and the external and pore surfaces are formed, at least in part, of the same organic polymer having some of the hydrogen atoms replaced with moieties selected from the group consisting of nitrogen atom, oxygen atom, amino group, hydroxyl group, carbonyl group, and carboxylic acid group, and wherein the external surface does not display surface roughness, according to SEM analysis, due to ablation of surface carbon atoms or chain scission.

Such a porous article can be used as a solid stationary phase for liquid chromatography, wherein the organic polymer has some of the hydrogen atoms replaced with functional groups selected from group consisting of amino, hydroxyl, carbonyl, and carboxylic acid.

In preferred embodiments of a solid chromatographic stationary phase, the amino, hydroxyl, carbonyl or carboxylic acid functional group is covalently linked, either directly or through a spacer group, to a chromatographically active group. Suitable chromatographically active groups include, without limitation, an anion exchanger, a cation exchanger, a hydrophobic group, a hydrazide group, a reactive

group for covalent bond formation through protein amino groups, a substituent group for reversed phase chromatography and a substituent group for chiral chromatography.

A spacer group may be placed intermediate the chromatographically active group and the base solid support. Suitable spacer groups include, without
5 limitation, a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase a fractal polymer phase, and a grafted polymer having a plurality of amine or hydroxyl groups. The spacer moiety may provide a pellicular structure on the surface of the porous article, where the porous article is preferably a monolith. While the pellicular structure distances the reactive site from the
10 solid support, it also may provide a favorable chemical environment for the chromatographic separation, *e.g.*, it may render the environment around the eluent and the solid support more hydrophilic.

In another aspect, the invention provides a device for liquid chromatography. The device includes a housing, where the housing includes a liquid
15 inlet, a wall member, and a liquid outlet. The liquid inlet is in liquid communication with a porous solid stationary phase having an external surface, a bulk matrix and pores extending from the external surface into the bulk matrix, wherein the pores define a pore surface. The bulk matrix is formed, at least in part, of an organic polymer comprising carbon and hydrogen atoms, and the external and pore surfaces are formed,
20 at least in part, of the same organic polymer having some of the hydrogen atoms replaced with functional groups selected from amino, hydroxyl, carbonyl, and carboxylic acid. The external surface does not display surface roughness due to ablation of surface carbon atoms or chain scission. The wall member is in substantially continuous contact with the external surface of the solid stationary phase and is affixed
25 thereto in a manner requiring that substantially all liquid flow proceeds axially through the solid stationary phase and substantially no liquid flow proceeds between the solid stationary phase and the wall member. The device also has the liquid outlet in liquid communication with the solid stationary phase and positioned substantially axially opposite the liquid inlet.

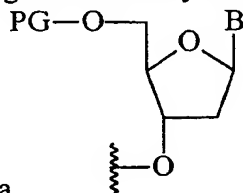
30 The solid chromatographic stationary phase may be monolithic, *i.e.*, a single continuous piece of porous polymer. In preferred solid stationary phases, the amino, hydroxyl, carbonyl or carboxylic acid functional group is covalently linked, either directly or through a spacer group, to a chromatographically active group. Suitable chromatographically active groups include, without limitation, an anion
35 exchanger, a cation exchanger, a hydrophobic group, a hydrazide group, a reactive group for covalent bond formation through protein amino groups, a substituent group

for reversed phase chromatography and a substituent group for chiral chromatography. When a spacer group is positioned intermediate the chromatographically active group and the base solid support, suitable spacer groups include, without limitation, a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase a fractal polymer phase, and a grafted polymer having a plurality of amine or hydroxyl groups. The spacer moiety may provide a pellicular structure on the surface of the porous article, where the porous article is preferably a monolith. While the pellicular structure distances the chromatographically active site from the solid support, it also may provide a favorable chemical environment for the chromatographic separation.

The invention also provides methods of chromatographically separating dissolved and/or suspended solutes and small articles (*e.g.*, cells) using the solid supports and/or device for liquid chromatography as described above.

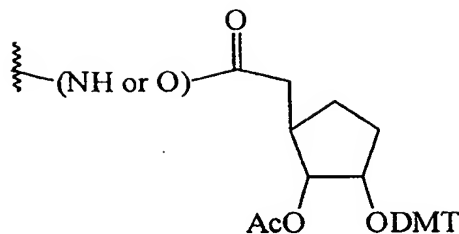
In another aspect, the invention provides a porous article suitable for solid phase biochemical synthesis, in which the porous article has an exterior surface, a bulk matrix and pores extending from the exterior surface into the bulk matrix, where the pores define an interstitial surface. The bulk matrix is formed, at least in part, of an organic polymer comprising carbon and hydrogen atoms, and the exterior and interstitial surfaces are formed, at least in part, of the organic polymer comprising carbon and hydrogen atoms wherein some of the hydrogen atoms are replaced with oxygen or nitrogen atoms. The exterior surface of the porous article does not display appreciable surface roughness, according to SEM analysis, due to ablation of surface carbon atoms or chain scission. The oxygen or nitrogen atoms are bonded, either directly or indirectly, to a protected nucleoside or amino acid. A preferred porous article is prepared from scintered non-porous polyolefin particles, and is in the form of a monolith.

The nitrogen or oxygen atoms may be directly or indirectly bonded to a



protected nucleoside of the formula

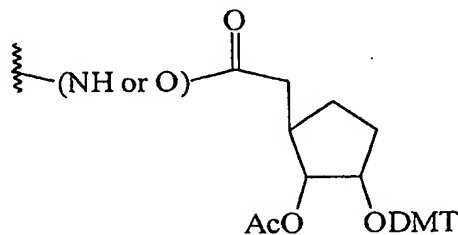
Alternatively, the nitrogen or oxygen atoms may be directly or indirectly



bonded to a universal support of the formula

In a preferred embodiment of an article used in solid phase biochemical synthesis, there is a spacer moiety positioned intermediate the nitrogen or oxygen atom and the protected nucleoside or amino acid. Suitable spacer moieties include a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, or a fractal polymer phase. The spacer moiety may be a grafted polymer having a plurality of amine or hydroxyl groups. The spacer moiety may provide a pellicular structure on the surface of the porous article, where the porous article is preferably a monolith. The spacer moiety may provide a pellicular structure on the surface of the porous article, where the porous article is preferably a monolith. While the pellicular structure distances the reactive site from the solid support, it also may provide a favorable chemical environment for the oligonucleotide chemistry of the invention, e.g., more hydrophilic.

Another aspect of the invention provides a universal support for solid



phase synthesis, having the structure

wherein the solid support is an insoluble organic or inorganic material which is inert to the reaction conditions employed for the solid phase synthesis. Preferably, the solid support is one of controlled pore glass beads, polystyrene beads, polystyrene/divinylbenzene beads, polystyrene/polyethylene glycol beads, polymeric membranes, polymeric films, or sintered polymeric particles.

The invention further provides a device for solid phase synthesis, where the device includes a porous article as described above positioned within a housing. The porous article is preferably in the form of a monolith, and contains a pellicular phase. The porous article, with or without the pellicular phase, may be attached to the universal support described above.

In yet another aspect, the invention provides methods, assay devices and kits useful for diagnosing and/or monitoring patient conditions or diseases. In one aspect, methods are provided for detecting a target molecule in a sample, comprising the steps of: (a) combining a sample with a diagnostic agent having affinity for a target molecule and immobilized on a surface-functionalized porous article, under conditions and for a time sufficient to allow the target molecule to bind to the diagnostic agent, wherein the porous article comprises at least one organic polymer and has (1) a bulk matrix, (2) an exterior surface and (3) pores extending from the exterior surface into the bulk matrix and defining an interstitial surface; wherein some hydrogen atoms present within the organic polymer of exterior and interstitial surfaces are replaced with one or more functional groups selected from the group consisting of amino, hydroxyl, carbonyl and carboxylic acid groups; and wherein the exterior surface does not display appreciable surface roughness, according to SEM analysis, relative to the surface of an unmodified articles; and (b) detecting the target molecule bound to the diagnostic agent, and therefrom detecting the presence of the target molecule in the sample.

Within one embodiment, the surface-functionalized porous article has a pore volume that is within 10% of the pore volume prior to surface functionalization. Within certain embodiments, the organic polymer is polyethylene and the surface-functionalized porous article comprises hydroxyl or amino groups on the exterior and interstitial surfaces. Spacer groups as described above may be placed intermediate the solid support and the diagnostically active functional group.

In another aspect, the present invention provides a kit for detecting a target molecule in a sample, comprising: (a) a diagnostic agent having affinity for a target molecule and immobilized on a surface-functionalized porous article, under conditions and for a time sufficient to allow the target molecule to bind to the diagnostic agent, wherein the porous article comprises at least one organic polymer and has (1) a bulk matrix, (2) an exterior surface and (3) pores extending from the exterior surface into the bulk matrix and defining an interstitial surface; wherein some hydrogen atoms present within the organic polymer of exterior and interstitial surfaces are replaced with one or more functional groups selected from the group consisting of amino, hydroxyl, carbonyl and carboxylic acid groups; and wherein the exterior surface does not display appreciable surface roughness, according to SEM analysis, relative to the surface of an unmodified articles; and (b) a detection reagent.

Within a further aspect, a microreactor array system is provided, comprising a two or a three dimensional array of porous solid microreactors, wherein each microreactor comprises a surface-functionalized porous article; wherein each

porous article comprises at least one organic polymer and has (1) a bulk matrix, (2) an exterior surface and (3) pores extending from the exterior surface into the bulk matrix and defining an interstitial surface; wherein some hydrogen atoms present within the organic polymer of exterior and interstitial surfaces are replaced with one or more functional groups selected from the group consisting of amino, hydroxyl, carbonyl and carboxylic acid groups; wherein the exterior surface does not display appreciable surface roughness, according to SEM analysis, relative to the surface of an unmodified articles; and wherein at least one two-dimensional array comprises a diagnostic agent immobilized on the surface of a surface-functionalized porous article; the porous solid microreactors being capable of receiving sample and reagent liquids and taking up the sample and reagent liquids by capillary action combined with gravity flow; each of the porous microreactors being separated from the other porous solid microreactors in an x-dimension and a y-dimension such that cross-talk between said porous solid microreactors is substantially eliminated.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth below which describe in more detail certain procedures or compositions of the invention, and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic cross-sectional view of a sidearm reactor according to the present invention.

Figure 2 is a cross-sectional view of the sidearm reactor of Figure 1, as seen along the lines 2-2.

Figure 3 is a diagram illustration exemplary amplification chemistry according to the present invention which may be used to introduce spacer group onto the solid support.

Figures 4 and 5 are cross-sectional view of cassette housings which are useful for incorporating functionalized solid supports of the invention.

Figure 6 is a flow chart that shows a method for modifying materials that have amino functionalities introduced by plasma treatment ("AFR-treated materials") with linear polyethylene glycol (PEG) moieties for use in applications described herein.

Figure 7 is a flow chart that shows a method for building dendritic molecules on AFR-treated materials, and their modification for DNA synthesis or anion exchange chromatography.

Figure 8 is a flow chart that shows a method for adding dendritic molecules on AFR-treated materials, and their modification for DNA synthesis or ion exchange chromatography.

Figure 9 is a flow chart that shows a method for attachment of StarPEG
5 onto AFR-treated materials.

Figure 10 is a flow chart that shows a method for chemical modification of PEGylated Materials for DNA synthesis and for anion exchange chromatography.

Figure 11 shows acrylamide gel electrophoresis and densitometry analysis of 15-base long oligonucleotides prepared on Nanolith, as described in
10 Examples 27.9 (16-61), 27.10 (16-62), and 27.11 (16-63).

Figure 12 shows an anion exchange chromatogram of 15-base long oligonucleotides prepared on Nanolith, as described in Example 27.10.

Figure 13 shows acrylamide gel electrophoresis of 15-base long oligonucleotides prepared on Nanolith (16-86; Example 27.10), TentaGel (16-87),
15 ArgoGel (16-88), and CPG (500 Å; 16-89).

Figure 14 shows the results of densitometry analysis of 15-base long oligonucleotides prepared on Nanolith (16-86; Example 27.10), TentaGel (16-87), ArgoGel (16-88), and CPG (500 Å; 16-89).

Figure 15 shows a trityl record (coupling efficiency) for a 75-base long
20 oligonucleotide prepared on Nanolith, as described in Example 27.10.

Figures 16A and 16B show a trityl record (coupling efficiency) for a 200-base long oligonucleotide prepared on Nanolith, as described in Example 27.10.

Figures 17A and 17B show a trityl record (coupling efficiency) for a 200-base long oligonucleotide prepared on 1000 Å CPG.

25 DETAILED DESCRIPTION OF THE INVENTION

The following section provides a description of some of the nomenclature that is useful in describing the solid supports of the present invention. Thereafter, there is a discussion of preferred methodology that may be used to functionalize an otherwise non-functionalized solid support (a "precursor porous
30 article"), to thereby introduce chemical handles and provide a "surface-functionalized porous article," where the chemical handles may be elaborated into functional groups through which solid phase synthesis may be initiated, to thereby provide a "modified porous article."

1. *Methods for Producing Porous Articles*

A. POROUS ARTICLES

The following general nomenclature is useful in describing the porous articles of this invention, including the precursor porous articles and the modified articles. Various portions of the surface of a porous article may be distinguished from one another depending on location, chemical reactivity, responsiveness to various analytic techniques, etc. For example, a porous article has an exterior surface, by which is meant the surface which is actually visible using a microscopic technique, such as scanning electron microscopy, without having to cut or section the article. The porous article contains pores, which extend from the exterior surface into the bulk matrix. The porous article thus has pore surfaces, which are essentially the surfaces that surround and define the pores of the article. The pore surfaces may sometimes be referred to as the interstitial surface because they surround the interstitial volume of the porous article, where the interstitial volume is all of the volume within the article that is not formed of organic or inorganic material.

Some fraction of the pore surface is visible using microscopy to examine the porous article, and thus the exterior surface includes a fraction of the pore surfaces, called the exterior pore surfaces. However, the majority of the pore surfaces are typically buried within the porous article, and are not visible by microscopy unless the porous article is cut or sectioned to reveal the internal pores. This major portion of the pore surfaces, which is not visible by scanning electron microscopy, is called the internal or interstitial pore surface of the porous article. Thus, the surface of a porous article includes an exterior pore surface and an internal or interstitial surface.

In terms of volume, the porous article has an interstitial volume, which is defined as essentially all of the volume within the article that would be accessible to a fluid that entered the article, *i.e.*, the volume surrounded by the pore surfaces which does not contain the organic or inorganic material that forms the article. The interstitial volume is also known as the void volume. The volume of the article which is formed of organic and inorganic material, and which does not include the surface of the article, is referred to herein as the article's bulk matrix, or alternatively the polymer matrix or the bulk volume of the article. The portion of the article's bulk matrix which is present at or within 1,000 angstroms of the surface will be referred to herein as forming the near surface region. The portion of the porous article that does not form either the interstitial volume, the surface or the near surface region will be referred to as the interior bulk matrix of the article.

Precursor porous articles useful in the invention are formed, in whole or part, of organic polymer, *i.e.*, carbon and hydrogen containing polymers. Preferably, the precursor article is formed entirely from organic polymer. However, the precursor article may be a composite of inorganic material and organic polymer. When the precursor article is a composite, then at least some of the surface of the article is formed of organic polymer, and preferably at least some of the bulk matrix is formed of the same organic polymer. A preferred composite article has a surface that is entirely formed of organic polymer, and more preferably has a bulk matrix that is predominately organic polymer.

Because the invention provides a very mild method of introducing functionality onto the surface of an organic polymer, essentially any organic polymer having hydrogen atoms may be treated according to the inventive method. The polymer can be a synthetic, semisynthetic, or naturally occurring organic polymer. For instance, the organic polymer which forms the precursor article may be a hydrocarbon. Preferred hydrocarbon polymers are formed from olefin, *i.e.*, the organic polymer is a polyolefin. Suitable hydrocarbon olefins from which the precursor article may be made include, without limitation, ethylene, propylene, butylene, butadiene, styrene, α -methylstyrene, divinylbenzene and the like. Hydrocarbon polymers are well known in the art, and are listed in, *e.g.*, *The Polymer Handbook*, 3rd Edition, Brandrup, J. et al. (eds.) John Wiley & Sons, Inc., New York, NY (1989). Articles formed in whole or part from polyethylene are preferred precursor articles, and more preferably the entire precursor article is formed of polyethylene.

The organic polymer which forms the precursor article may contain heteroatoms such as halogen, silicon, sulfur, oxygen and/or nitrogen atoms, as present in, *e.g.*, polyamide, polyvinylchloride, polyurethane, polyacrylate, phenolic resins, alkyd resins, polyester, cellulose, starch, polysaccharide, polypeptide, rubber, gutta percha, poly(ethylene terephthalate), polyimide, polytetrafluoroethylene, poly(vinylidene fluoride), polysulfone, poly(ether sulfone), Kynar[™], PEEK, Tefzel[™], Teflon[™], polydimethylsiloxane, polyphenylsiloxane, dimethylsiloxane/methylvinylsiloxane, copolymer, substituted polyphosphazenes, and the like. When heteroatoms are present in the polymer's repeating unit, the repeating unit preferably contains more carbon atoms than non-carbon atoms (excluding hydrogen and halogen atoms). Again, polymers containing heteroatoms which may be used according to the present invention are well known in the art, and are listed in, *e.g.*, *The Polymer Handbook* (*supra*).

Essentially, the organic polymer which forms the precursor article may have any molecular weight, molecular weight distribution, stereochemical configuration

or conformation, degree of crosslinking, density, tensile strength, extent of entanglement, glass transition temperature (although this should be above room temperature, so that the article is a solid at room temperature), tacticity, melt viscosity and color, among other properties that characterize polymers.

5 Preferably, the organic polymer which forms the precursor article has a relatively high molecular weight, on the order of 1,000,000 daltons or more (number average molecular weight). When the polymer has a relatively high molecular weight, there tends to be a greater degree of entanglement among the polymer chains. This greater degree of entanglement tends to inhibit the polymer chains from moving around
10 within the article. Consequently, functionality which is initially introduced to the surface of a porous article will tend to remain at that surface for a longer period of time when the average molecular weight of the polymer chains is relatively high.

 As stated above, polyethylene is a preferred polymer which forms the bulk matrix of the inventive article. So-called ultra-high molecular weight polyethylene
15 (UHMWPE) is a preferred polyethylene. UHMWPE is described in, *e.g.*, U.S. Patent No. 5,531,899. Polyethylene is typically characterized in terms of its density, and both high density and low density polyethylene may be used to form the precursor article.

 The article which is functionalized according to the present invention is porous. Preferably, the article is characterized by a plurality of interconnected pores on
20 a larger-than-molecular scale. The article may also be described as permeable, in that the pores are interconnected to allow fluid flow from an exterior surface into an adjacent region in the structure, and from one adjacent region to another. Preferably, the pores are interconnected so that fluids may flow throughout the article. The pores may be isotropic or anisotropic, and may be nondirectional or unidirectional, to name a
25 few of the ways in which porous articles may be characterized. The pores may be connected by a so-called tortuous path, which essentially means that the pore channels are randomly directed through the article in a non-directional manner.

 However, the pores need not be interconnected but instead may be separate from one another. For example, the pores may be separate from one another
30 but extend completely through the article, or the pores may terminate within the porous article, where the later situation provides for so-called "non-through" pores. However, the pores should be accessible to fluids that contact the exterior surface of the porous article, *i.e.*, the porous article should not be a closed cell foam or the like where all of the pores are isolated from the exterior surface.

35 The porosity of the article may be characterized in terms of pore diameter and void volume. The pores generally have effective diameters larger than

about 0.01 microns (1 angstrom), preferably at least about 1 micron up to about 2000 microns, and more preferably from about 10 to about 1000 microns. Thus, the porous article can be distinguished from semipermeable solid-phase membranes which generally have intermolecular openings on a molecular size scale for molecular diffusion. The porous article may be microporous, in which case the article has a pore size in the range between about 0.05 and about 20 microns. The porous article may be macroporous, in which case the article has a pore size of about 20 microns to about 2000 microns, preferably about 20 to 500 microns. A preferred porous article has an effective pore size of about 1 to about 50 microns. Porosity may be determined according to ASTM D276-72, and pore size distribution may be determined according to ASTM F316-70.

The porosity of the article can also be characterized in terms of pore volume. Thus, the porosity may be defined as the pore volume divided by the total volume of the article. In percentage terms, porosity refers to the pore volume as defined above, multiplied by 100. While modified porous articles of any pore volume from 1% to 90% may be used in the present invention, preferred modified articles have a pore volume of about 20% to about 80%, more preferably have a pore volume of about 30% to about 70%, and still more preferably have a pore volume of about 35% to about 60%.

Porous articles may be made by methods which are well known in the art. The variety of techniques which are described in Kesting, Robert E., *Synthetic Polymeric Membranes*, John Wiley & Sons, New York, 1985, pp. 237-309, are exemplary. The pores may be introduced by mechanical perforation, by the introduction of pore producing agents (*e.g.*, porogens) during the matrix forming process, through various phase separation techniques, or other methods. Indeed, the particular source of the porous article is not especially important to the practice of the present invention. However, the properties of the porous material should be selected so that the article has the necessary porosity, strength, durability and other properties which render it suitable for use as a support for solid phase synthesis. The porous article is preferably self-supporting.

A porous article prepared by sintering together finely divided polymer particles (*i.e.*, particulated polymer) has been found to have very good physical properties for use as a solid support for organic and biomolecule synthesis. *See, e.g.*, U.S. Patent No. 3,051,993. The polymer particles may or may not be porous in and of themselves. However, the material formed by sintering the polymer particles will have void volume between the sintered particles, where that void volume creates interstitial surface. Such materials are referred to herein as sintered articles, *e.g.*, sintered

polyolefin if the polymer particles are made of polyolefin. A porous material prepared by sintering non-porous polyolefin particles (typically beads) is a preferred porous material according to the invention, and for articles made from polyethylene, is commercially available from, *e.g.*, Porex Technologies USA (Fairburn, GA) under their trade designation POREX™.

Precursor and modified porous articles may have essentially any shape. For example, the article may be in the shape of a cube, block, sphere, tube, rod or cylinder, sheet, disc, membrane, film, monolith or the like. Articles in the form of a block/cube or rod/cylinder are preferred for many solid phase synthesis applications, and may be obtained from Porex Technologies USA (Fairburn, GA). A preferred article in the form of a film is porous polyethylene film, *e.g.*, CELGARD™ K-878, which is commercially available from Celanese Corporation (Norristown, NJ). Microporous polymeric films which may be treated according to the invention are described in, *e.g.*, U.S. Patent Nos. 3,839,516, 3,801,404, 3,679,538, 3,558,764, and 3,426,754.

The article may be a composite of two polymers. An exemplary composite is a breathable, hydrophobic polysiloxane membrane reinforced with poly(tetrafluoroethylene) which is sold under the tradename SILON™ by Bio-Med Sciences, Inc. (Bethlehem, PA). These membranes are typically used as wound dressings, gas and fluid exchange membranes, etc. The article may have a shape resulting from drawing, molding, sintering, or other polymer processing step.

B. REMOTE RADICAL FORMING CONDITIONS

A precursor porous article as described above may be exposed to gas phase radicals under remote conditions, to introduce chemical handles to the surface of the article. Those chemical handles, which are either nitrogen atoms (as present in, *e.g.*, amino groups) or oxygen atoms (as present in, *e.g.*, hydroxyl groups) may be elaborated into functional groups useful in solid phase synthesis, to thereby provide a modified porous article of the invention.

The solid phase synthesis supports of the present invention are preferably prepared by contacting a precursor solid support (hereinafter, "the specimen") with reactive gas-phase radicals. The radicals impart functionalization to the solid support, and are particularly well-suited to providing functionalization throughout the entire surface (including the innermost pore surfaces) of a porous article.

The following describes one suitable approach to providing an environment of gas-phase radicals which may be used to functionalize an organic or organic/inorganic hybrid polymer. The radicals are generated from a source gas, in a

non-equilibrium, low pressure environment, and delivered to the specimen by at least one of convective and diffusional transport. The radicals are generated from the source gas by exposing the source gas to ionizing conditions as created by, for example, a radio frequency (RF), microwave or direct current discharge (any of which will create a gas plasma discharge), laser sustained discharges, UV laser photolysis, high-powered
5 UV/VUV lamp driven photolysis, high energy electron beams, and other high-intensity ionizing radiation sources. While any of these ionizing conditions may be used to generate the reactive radicals, gas plasma discharge is a preferred technique.

The source gas comprises oxygen, ammonia, or a mixture of nitrogen
10 and hydrogen. Preferably, oxygen and ammonia are not used simultaneously, and the mixture of nitrogen and hydrogen is not used with either oxygen or ammonia. These source gases may be used in pure form (*i.e.*, above about 95% purity) or in dilute forms, where suitable diluent gases include helium, argon and nitrogen. Diluted source gases afford the advantage that reactive radicals that are generated from dilute oxygen,
15 nitrogen or ammonia demonstrate fewer recombination events, thus providing for a longer-lasting and higher concentration of reactive radicals in the reactor. In addition, the energy transferred from an excited metastable species formed from a diluent gas, *e.g.*, Ar*, can increase the yield of the reactive radical.

For example, 10% oxygen in argon at a pressure of 2 Torr provides about
20 the same ability to functionalize a porous specimen as does pure oxygen. Argon is a preferred diluent gas because the metastable electronic excited state of argon promotes dissociation of molecular oxygen or ammonia by collisional energy transfer, and so promotes in-plasma dissociation kinetics. Thus, dilute gases having about 10% concentration of oxygen or ammonia are preferred according to the invention.

When oxygen serves as the source gas, the oxygen (O₂) is converted into
25 atomic oxygen radical (O₁). With the reactor and operating conditions as described herein, the minimum concentration of atomic oxygen radical within the reactor (at the location where the atomic oxygen contacts the specimen, and at 298°C) is preferably at least 1×10^{13} atoms/cc, as measured by NO₂ chemiluminescent titration (as described in,
30 *e.g.*, Kaufman, F., *Progress in Reaction Kinetics*, vol. 1, Pergamon Press, London, pp. 1-39, 1961). In addition to atomic oxygen, hydroxyl radicals will also typically react with the specimen. This is due to the fact that one possible reaction that can occur when atomic oxygen contacts the specimen is the formation of hydroxyl radical. This hydroxyl radical is then available to react with the specimen. In one embodiment of the
35 invention, a "sacrificial" polymer may be placed upstream of the specimen, in order to enhance the concentration of hydroxyl radicals that react with the specimen.

When ammonia serves as the source gas, two reactive radicals are generated: hydrogen (H_1) and amino (NH_2). While not intending to be bound by any theory, it is believed that hydrogen radicals initially react with the specimen to abstract a hydrogen radical and thereby form hydrogen gas. The residual radical site on the specimen then reacts with an amino radical to place an amino group on the specimen. In any event, with the reactor and operating conditions as described herein, the minimum concentration of atomic hydrogen radical within the reactor (at the location where the atomic oxygen contacts the specimen) is preferably at least 1×10^{16} atoms/cc at a temperature of $298^\circ C$, as measured by NO chemiluminescent titration (as described in Kaufman 1961, *supra*) and calculated from the pressure rise seen on ignition of the plasma source. This minimum value is preferably maintained regardless of the working pressure.

It is often the case, particularly when using oxygen as the source gas, that undesirable by-products are produced along with the desired gas-phase radicals, where the by-products may be undesirable because they can cause damage to the specimen. Such by-products include electrons, ions and UV radiation. Thus, the preferred reactor provides an environment which precludes, or at least substantially precludes, contact between the specimen and undesirable by-products. Preferably, the reactor allows the undesirable by-products to undergo natural decay prior to their contacting the specimen. In other words, the preferred reactor and operation thereof provides that a specimen is exposed to an environment wherein the rate of decay of desired gas-phase atomic and molecular radicals is much less than the decay rate of undesirable by-products. This environment provides for "remote" treatment of a specimen using radical forming conditions.

The terms "remote plasma", "downstream plasma", and "flowing discharge" are generally synonymous. Techniques to achieve remote plasma discharge are described in, e.g., Foerch, R. et al. "A comparative study of the effects of remote nitrogen plasma, remote oxygen plasma, and corona discharge treatments on the surface properties of polyethylene" *J. Adhesion Sci. Technol.* **5**(7):549-564 (1991); Deshmukh S.C. et al. "Remote plasma etching reactors: modeling and experiment" *J. Vac. Sci. Technol. B*, **11**(2):206-215 (Mar/Apr 1993); Boitneott, C. "Downstream plasma processing: considerations for selective etch and other processes" *Solid State Technology*, pages 51-58, October 1994; Brady J.P. et al. "A comparative study of the effects of ammonia and hydrogen downstream plasma treatment on the surface modification of polytetrafluoroethylene" *Polymer* **37**(8):1377-1386 (1996).

As used herein and in the above publications, the terms "remote plasma" and "downstream plasma" generally refer to an environment wherein the specimen being modified by the plasma is located such that the specimen is not exposed to plasma-derived species that can cause collateral damage to the specimen. Remote or downstream plasma techniques (hereinafter, simply "remote plasma") described in the literature, which are directed, for example, to etching processes in the semiconductor industry, or otherwise modifying the surface of a substrate, may be used in the process of the present invention so long as they achieve little (less than 10% mass loss) or essentially no (less than 1% mass loss) collateral damage to the specimen while achieving complete functionalization of the surface of the porous article. Complete functionalization of all pore surfaces, both internal and external (external pore surfaces may be seen from visual examination of the exterior surface of the porous article), occurs when all pore surfaces, both internal and external, have been modified in the desired way and to a useful extent as described herein.

Because there is so little mass loss, there is little or no deviation in the pore size as measured on the surface of the article, compared to the average diameter of the pores within the matrix of the material (assuming, of course, that there was little or no deviation in the pore size of the precursor specimen). Thus, porous articles having essentially the same pore diameters throughout the article, as well as articles having average pore diameters that deviate by no more than 1% or 2% between the exterior surface and the interior matrix, can be prepared according to the invention.

While remote plasma techniques described in the literature typically separate the specimen being treated from direct contact with the glow discharge plasma, it is not always the case that the specimen is spared contact with light that is emitted from the glowing discharge. In remote treatment according to a preferred embodiment of the present invention, photons emitted from the excited gas, and particularly UV radiation, do not contact the porous article. Thus, the yellowing of a specimen that often accompanies direct treatment of a organic polymer is not observed in remote treatment as described herein.

A preferred reactor, for process optimization studies, which may be used to produce a remote plasma environment is the atomic oxygen reactor having at least one sidearm conduit, which is described in U.S. Patent No. 5,332,551 to Steven L. Koontz. When operated as described herein, this atomic oxygen reactor is used to achieve flowing discharge radical chemistry (FDRC) to modify the surfaces of porous articles. A preferred embodiment of an atomic oxygen reactor having at least one sidearm conduit (hereinafter, a "sidearm reactor") is illustrated in Figures 1 and 2 set

forth herein, and may be operated as described below. Further description of the sidearm reactor and its operation appears in Koontz, S.L. et al. "The reaction efficiency of thermal energy oxygen atoms with polymeric materials", *Materials Degradation in Low Earth Orbit*, Srinivasan V. et al. eds., pages 189-205, The Minerals, Metals & Materials Society (1990) (Published proceedings of a symposium sponsored by the TMS-ASM joint corrosion and environmental effects committee, held at the 119th annual meeting of The Minerals, Metals & Materials Society, February 17-22, 1990).

As shown in Figure 1, a sidearm reactor 10 comprises a longitudinally oriented flow chamber 12 formed from three sections, namely an inlet section 14, a mid-section 16 and an exhaust section 18. These sections are secured together using conventional coupling flanges 20, 22, 24, and 26. Glass is a preferred material from which to construct the flow chamber 12. The reactor 10 further comprises an inlet line 28 through which the precursor to the reactive gas-phase radical (also termed a "source gas") may be introduced to the discharge generating apparatus 32. The apparatus 32 generates a plasma discharge that converts the source gas into a collection of reactive gas-phase radicals, as well as undesirable byproducts (collectively a flowing discharge gas). The flowing discharge gas exits the discharge generating apparatus 32 through the line 30 and passes into the inlet section 14 of the flow chamber 12.

The discharge generating apparatus 32 comprises a power supply and a cavity configuration wherein the source gas is exposed to a discharge. The cavity should afford a configuration of an electric field such that conducting surfaces are configured to produce a standing wave EM field. Suitable microwave discharge cavities are described in, e.g., Fehsenfeld, F.C., et al. *The Review of Scientific Instruments* 36(3):294-298 (March 1965). Suitable power supplies include any radio frequency (RF), microwave or direct current power supplies. A suitable microwave power supply may be obtained from, for example, Raytheon Corporation, and identified under their trade designation as a Raytheon PGM-10. The Raytheon PGM-10 may be used in conjunction with an Evensen-type discharge cavity. The apparatus 32 may also consist of an ASTEX S-250 microwave power supply in conjunction with an ASTEX DPC24 plasma head which creates a cavity wherein the plasma is formed. The ASTEX components are available from Applied Science and Technology, Inc., Woburn, MA. Either of these configurations may be used with any source gas of the invention. These power supplies are typically operated at (FCC-approved) 2.450 MHz, although the FCC also approves the use of 13 MHz band for industrial processing, and this could also be employed in the present invention.

Along the mid-section 16, and extending transversely to the longitudinal axis of the flow chamber 12, are one or more sidearms 38. The six sidearms of an exemplary reactor 10 are shown in Figure 2. At the distal end of each sidearm 38 is a specimen holder 40 which holds the specimen 49 to be modified by the reactive gas-phase radicals. The sidearm 38 also comprises a sidearm conduit 42 which provides fluid communication between the specimen holder 40 and the mid-section 16 of the flow chamber 12. The sidearm conduit 42 is preferably constructed from glass.

The specimen holder 40 can be made by modifying a CAJON brand union fitting (Cajon Company, Macedonia, OH). Drilling can be used to remove the internal lips of the fitting while leaving the O-ring vacuum seals 44, 46 at either end of a sleeve member 48 adjacent the conduit 42. Each specimen holder 40 may also include a specimen heater well 50 and a thermocouple well 52. The specimen 49 which undergoes surface-modification is placed at the distal end of the conduit 42, inside the sleeve member 48.

The exhaust section 18 of the flow chamber 12 is in fluid communication with a vacuum pump 34 via line 36 positioned therebetween. Optionally, a catalytic atomic oxygen or atomic hydrogen probe (shown by feature 58) may be positioned within the discharge section 18, to thereby measure the concentration of the atomic oxygen exiting the flow chamber 12. A suitable catalytic atomic oxygen probe may be prepared by wrapping fine silver wire or thin foil around a low thermal rated type J or K thermocouple probe (from, *e.g.*, Omega Scientific). A palladium probe may be used to measure hydrogen. A capacitance monometer connection (shown by feature 56) may also, or alternatively, be connected to the discharge section 18 in order to measure the pressure within section 18.

The reactor 10 is constructed and operated so that no significant radial concentration gradients of reactive gas-phase radicals exist within the sidearms. This is achieved, in part, by positioning the specimens within sidearms so that they are not subject to the direct flow of the glow discharge plasma generated by the discharge generating apparatus. The specimens are thus positioned so as not to be in direct contact with the plasma and so that photons from the plasma cannot reach the specimen.

The environment within the sidearms is preferably selected to deliver a uniform dose of reactive gas-phase radicals to the specimen(s). The term "uniform dose" means that the surface flux of radicals is essentially constant across the diameter of a sidearm. A uniform dose typically does, but need not, achieve a uniform spacing of induced functionality across the surface of the specimen. In addition, the environment should be such that the gaseous reactive radicals are able to diffuse to the surface of a

specimen, and diffuse into the pores of a porous specimen. In this way, the radicals are able to penetrate deeply into the interstitial volume of a porous specimen. Such an environment can be used to provide for variable (low to high) levels of surface functionalization, with a uniform distribution of the functionality across the surface.

5 Concurrent with surface functionalization, the sidearm environment minimizes, and may essentially eliminate, specimen degradation. Thus, the inventive process introduces functionality to the surface of a porous article while causing very little change in the pore volume of the article. Thus, surface-functionalized porous articles may be prepared having pore volumes which are within 10% of the pore volume
10 of the precursor porous article, and are preferably within 5% of the initial pore volume. When ammonia is the source gas, surface-functionalized porous articles may be prepared having pore volumes which are within 5% of the pore volume of the precursor porous article, and are preferably within 1% of the initial pore volume.

This lack of surface degradation is a desirable feature of the present
15 invention. Known methods to introduce functionality to a polymer, such as direct plasma treatment or solution-phase chemical oxidation, typically cause a substantial amount of chain scission and ablation of carbon atoms at the surface of the article. These effects are readily seen by scanning electron microscopy, where they give rise to surface roughness. Remote treatment with gas-phase radicals as described herein
20 achieves surface functionality throughout the entire surface of a porous article, while not causing much, if any, change in the morphology of the surface. Thus, in functionalized porous articles prepared by, *e.g.*, remote plasma discharge, the pore surface appears substantially the same as the exterior surface, as viewed by scanning electron microscopy.

25 Remote plasma treatment is achieved by proper selection of the reactor, including reactor dimensions and the materials from which the reactor is constructed, and proper selection of reactor operating conditions. These selections are not independent, and thus one selection must be made in view of the other. The following guidelines are useful in developing reaction conditions useful for achieving remote
30 plasma discharge.

Ultimately, in order to provide a uniform flux of reactive gas-phase radicals across the surface of a specimen, the selections are made so that the diffusional relaxation time (a characteristic time for gas diffusion to eliminate gas radical concentration gradients) of the reactive radicals within the sidearms is much smaller
35 than the characteristic decay time(s) of the gas-phase radicals as resulting from the sum of all surface and gas-phase loss processes. These diffusional relaxation and decay

times will depend on the dimensions of the sidearms, the reactivity of the materials from which the sidearms were constructed, the reactivity of the specimen, the total working pressure within the reactor, as well as the temperature within the reactor.

The upper and lower limits on the working gas pressure are determined by the need to provide enough of the specific reactive gaseous radicals of interest to complete the desired surface chemistry on the specimen in a reasonably short length of time. A reaction time of 1 minute or so, for a sample that is about 1 cm thick, is typical, although this time is highly dependent on the porosity of the specimen, and the average diameter of the pores. Highly porous samples, with small pore diameters, which characterizes many membranes, may require a reaction time on the order to 60 minutes or so. If the pressure is too high, for instance more than about 10 Torr (1300 Pa), then gaseous radical recombination reactions proceed at an undesirably fast rate, and rapidly deplete the reactive radical population. If the pressure is too low, say less than 0.1 Torr, then the number of gas phase radicals in the sidearm is too low to be practically useful. A working gas pressure between about 0.1 and 10 Torr (roughly 10 to 1300 Pa) is thus practically useful, with gas pressures of about 0.5 to 5 Torr (roughly 50 to 500 Pa) being preferred regardless of the source gas.

An important factor limiting the use of higher pressures is the increasing heat capacity of the gases at higher pressures. It is known that plasma discharges at atmospheric pressure produce high enthalpy gas streams which may vaporize metals and destroy polymers via purely thermal processes. Accordingly, as the pressure increases inside a reactor according to the invention, the temperature of the flowing gases increases, rapidly approaching the thermal limits of the apparatus. Operating conditions and pressures which would expose the specimen to temperatures in excess of 200°C are generally not preferred. Preferred temperatures around the specimen are described below. As the effect of pressure on heat capacity depends to some extent on the identity of the source gas, the optimal working pressure is dependent on the choice of source gas.

The upper and lower limits on the working temperature within the sidearm are essentially determined by balancing desired reaction rate with rate of undesired specimen decomposition (decomposition occurs, for example, by ablation of carbon atoms from the polymer, typically with the formation of carbon dioxide). For oxygen source gas, a temperature of about 300 K affords a desired reaction rate that is much greater than the decomposition rate, and a typical specimen will undergo complete reaction in a matter of minutes. As the working temperature is lowered, the desired reaction rate is even more favored in comparison to the decomposition rate, (the

decomposition rate becomes very slow), however the desired reaction rate also slows down, and thus lower temperature necessitate longer reaction times to prepare the desired sample. At higher temperatures, full functionalization of a specimen is more rapid, however specimen decomposition becomes noticeable. Accordingly, a working
5 temperature of less than about 350 K is preferred, with a temperature range of about 250 K to about 325 K being more preferred, and a temperature range of about 275-310 K being still more preferred, when oxygen is the source gas.

When ammonia is the source gas, the activation energy for sample decomposition is very much greater than the activation energy for the desired reaction.
10 Accordingly, ammonia allows for a wider latitude in the selection of the working temperature because the rate of the desired reaction will almost always be much greater than the rate of the undesired decomposition reactions. Thus, when ammonia is the source gas, relatively high temperatures are desirably used, for the reason that preparation of the fully functionalized specimen is completed more rapidly. A working
15 temperature of about 275 K to about 400 K is preferred when using ammonia as the source gas, with a working temperature of about 300 K to about 375 K being more preferred, and a working temperature of about 325 K to about 350 K being still more preferred. While temperatures higher than 400 K can be used with ammonia, very little increase in the desired reaction rate is observed compared with using 300 K, and thus
20 for reasons of economy and safety, a lower temperature is preferred. At temperatures lower than about 275 K, the rate of specimen functionalization using ammonia becomes undesirably slow from a commercial point of view.

The dimensions and configurations of a practical commercial reactor to accomplish remote plasma discharge reaction are selected while keeping the above
25 temperature and pressure limits in mind, and can be designed using process data generated with the side-arm reactor. Thus, the convective flow time between the end of the plasma zone (feature 30 in Figure 1) and the beginning of the remote plasma treatment section (feature 32 in Figure 1) is preferably greater than 1×10^{-4} seconds when the operating pressure is on the order of 1-2 Torr. Adjustments to the total mass
30 flow rate of the gas and the pumping speed of the vacuum system allows variation of both the total pressure within the reactor and the residence time of the radicals. In general, as the mass flow rate is increased for a constant pump speed, the convective flow time of the radicals increases and the pressure increases. However, as the pump speed is decreased while the mass flow rate is kept constant, the pressure increases
35 while the convective flow time also increases. Suitable operating conditions and reactor dimensions include an operating pressure of 1-2 Torr, an operating temperature of 298-

325 K, a sccm of 125-135 and a distance of about 4 inches (about 10 cm) from the end of the plasma zone to the beginning of the remote plasma treatment section.

Given these pressure and temperature ranges, the diameter of the sidearm may be selected based on the equations that predict the diffusional relaxation and chemical recombination times of radicals within a sidearm. As stated above, the sidearms 38 are constructed and operated so that no significant radial concentration gradients exist and a uniform dose of reactive gas-phase radical is thereby delivered to the specimen surface 49 which is held or secured transversely in the specimen holder 40. The elimination of radial concentration gradients in the sidearms 38 is determined according to well known gaseous chemical kinetic analysis, by determining the relative magnitude of two characteristic relaxation times, T_{diff} and T_{rcm} , where T_{diff} is the characteristic diffusional relaxation time for the sidearm 38 and T_{rcm} is the time required for all reactive radicals in a sidearm to recombine. When T_{diff} is much less than T_{rcm} , (e.g., $T_{diff} > 0.1 T_{rcm}$), then no significant radial concentration gradients exist and a uniform dose of reactive radical is delivered to the specimen surface.

T_{diff} is determined according to the equation:

$$T_{diff} = R^2/D$$

wherein R is the radius of the sidearm 38 and D is the diffusion coefficient of the reactive radical (about $120 \text{ cm}^2/\text{s}$ in air at 65 Pa when the reactive radical is atomic oxygen). Diffusion coefficient values for other gases and pressures may be obtained from the published literature, and/or calculated based on basic gas kinetic theory.

The chemical recombination (relaxation) time, T_{rcm} , is determined according to the equation:

$$T_{rcm} = R/r_c v$$

wherein r_c is the fraction of reactive radicals which recombine or are otherwise lost upon striking the sidearm surface. A description of radical recombination may be found in, e.g., Smith, W.V. "The surface recombination of H atoms and OH radicals" *J. Chem. Phys.* 11:110-124 (March, 1943) and Krongelb, S. et al. "Use of paramagnetic-resonance techniques in the study of atomic oxygen recombinations" *J. Chem. Phys.* 31(5):1196-1210 (November, 1954). More generally, r_c is the probability of the loss of a reactive radical from the population of reactive radicals, due to any first order or pseudo first order process. Such first order and pseudo first order processes include an atom or radical striking the sidearm wall or specimen within the sidearm, as well as the recombination of reactive radicals. The value of r_c is about 3.2×10^{-4} in the case of atomic oxygen, and 2×10^{-5} for hydrogen, with a sidearm constructed from glass. The

parameter "v" is the mean thermal speed of the reactive radical (about 6.3×10^4 cm/s at 300 K for atomic oxygen, about 6.5×10^4 cm/s at 300 K for ammonia and about 25.2×10^4 cm/s at 300 K for hydrogen). Thus, when $R = 1$ cm and the reactor is operated at 65 Pa and 300 K, T_{diff} is about 0.008 seconds and T_{rcm} is about 0.1 seconds for atomic oxygen, so that T_{diff} is less than $0.1 \times T_{rcm}$.

The sidearm reactor as described herein can be used to provide a predetermined, uniform dose rate of reactive radicals across a specimen surface. The rate at which a specimen is functionalized depends on the surface flux of the reactive radicals. Dose rate can be estimated by analytical solution of the following partial differential equation describing the diffusional transport and first order or pseudo-first order reactive radical reaction processes:

$$k_c C(r, z) = D \frac{1}{r} \frac{\partial}{\partial r} r C(r, z) + \frac{\partial^2}{\partial z^2} C(r, z)$$

wherein k_c is the rate constant for loss of reactive radical from the gas in the sidearm conduit from all first order processes, C is the concentration of the reactive radical, r is radial position of the specimen from the longitudinal axis of the sidearm conduit, and z is an axial position (distance from the sidearm conduit entrance to the main gas chamber) with the boundary conditions:

$$C(r, z) = C_o, \text{ at } z = 0; \text{ and}$$

$$-D \frac{\partial}{\partial z} C(r, z) = k_s C(r, z), \text{ at } z = z_1 \text{ (the specimen surface);}$$

wherein k_s is the rate constant for loss of reactive radical at the specimen surface.

If $T_{diff} < T_{rcm}$, the partial differential equation simplifies to:

$$\frac{k_c C(z)}{D} = \frac{\partial^2}{\partial z^2} C(z).$$

A general solution to this has the form:

$$C(z) = A \exp\left[-(k_c / D)^{0.5} z\right] + B \exp\left[(k_c / D)^{0.5} z\right]$$

wherein A and B are constants determined by application of the boundary conditions as follows:

$$A = \frac{G_B C_O}{G_A + G_B};$$

$$B = \frac{G_A C_O}{G_A + G_B};$$

wherein:

$$G_A = \left[D(k_c / D)^{0.5} - k_s \right] \exp \left[-(k_c / D)^{0.5} z_1 \right]; \text{ and}$$

$$G_B = \left[-D(k_c / D)^{0.5} + k_s \right] \exp \left[(k_c / D)^{0.5} z_1 \right].$$

The complete analytical solution for the case of no radial concentration dependence and first order reactive radical loss processes is as follows:

$$C(z) = \frac{C_o}{(G_A + G_B)} \left\{ G_B \exp \left[-(k_c / D)^{0.5} z \right] + G_A \exp \left[+ (k_c / D)^{0.5} z \right] \right\}$$

The reactive radical dose rate can thus be estimated, and it is readily appreciated that the dose rate can be increased dramatically by increasing the concentration of the reactive radical at the sidearm conduit entrance (at $z = 0$), and affected by the material used to form the sidearm conduit and the specimen material. Total reactive radical doses of about $10^{24} - 10^{26} \text{ cm}^{-2} \text{ day}^{-1}$ or more can be obtained with the reactor disclosed herein.

Using the reactor and operating conditions as described above, (specifically, 2 Torr total system pressure of aviators breathing oxygen as the source or working gas), an exposure time on the order of seconds (100-1,000) is typically sufficient to achieve essentially complete surface functionalization of a porous polyethylene disc (having, *e.g.*, a thickness of 1/16 inch (1.6 mm) and a diameter of 0.75 inch (19 mm) and a nominal pore size on the order of 20 microns). Reducing the exposure time can provide a partially functionalized specimen. However, degradation of such a disc using remote plasma treatment according to invention requires an exposure time on the order of hours (1-10) to achieve even a 5% mass loss. Thus, functionalization of even the innermost interstitial regions of a porous specimen is much faster than degradation of the specimen, using remote plasma treatment as described herein.

C. SURFACE-FUNCTIONALIZED POROUS ARTICLES

When gas-phase radicals as described above reach the precursor article and diffuse through the pores of the article, the exterior and interstitial surfaces become modified with functional groups such as amino, hydroxyl, carbonyl and carboxyl groups, and thereby form a surface-functionalized porous article. The modification is the result of replacing hydrogen atoms that are part of the basic structure of the porous article, with oxygen or nitrogen atoms, to form hydroxyl/carbonyl/carboxylic acid groups or amino groups, respectively, depending on the identity of the source gas. The functional groups which are added to a surface according to the remote gas-phase radical processes as described above are collectively referred to as "chemical handles."

The organic polymer(s) which form the precursor porous article are reactive with gas phase radicals, and will typically experience mass loss upon exposure to the radicals over an extended period of time. Mass loss typically occurs upon chain scission and subsequent ablation of carbon atoms. However, the polymer and treatment conditions are selected so that the rate of mass loss is relatively low compared to the rate at which functionality is introduced to the surface of the article. Accordingly, the polymer matrix, and particularly the interior bulk matrix of the article, is essentially unchanged by the treatment, and the supporting structure retains its bulk and mechanical properties. The remote gas-phase radical treatment described above thus avoids contact between the article and high energy beams which have been used in the art to create reactive sites at the surface, and in the bulk matrix. Such beams typically cause chain scission within the bulk matrix of the article, thereby weakening the mechanical strength of the article. The remote process as described above avoids this undesirable result, and provides an article with a bulk matrix that has not been modified by chain scission. Accordingly, modified porous article which is used in solid phase synthesis likewise has the same mechanical strength as the precursor porous article.

Generally, the diffusion of the gas-phase radicals from the exterior surface through the interstitial volume of the article proceeds in a distinct front. Behind the front, the interstitial surfaces of the pores become functionalized by the introduction of amino, hydroxyl, etc. groups. Ahead of the front, the pore surface retains its initial structure and functionality. When the reaction front is allowed to pass through the entirety of the article, the entire surface of the article gains functional groups.

The surface-functionalized porous article according to the present invention may be characterized in several way. For example, scanning electron microscopy (SEM) may be used to monitor the structural effects of the remote gas-phase radical treatment on a solid support. Typically, complete surface

functionalization of a porous polymer article can be achieved with no appreciable change in the surface morphology, where no appreciable change means that no change in morphology is observed as determined by scanning electron microscopy at a magnification of 50X, and preferably less than 2000X. However, if the precursor porous article is exposed to an extended treatment with remote plasma discharge, one can observe some mass loss by SEM. For example, one can use SEM to determine if the treatment has modified the appearance of the pores on the exterior surface of the solid support. However, the average pore size of the surface-functionalized porous article is essentially the same as in the precursor porous article that serves as the starting materials. Furthermore, the bulk density of the surface-functionalized porous article is essentially the same as the bulk density of the precursor article. The porous nature of the precursor article is essentially unchanged by the remote gas-phase radical process described herein, except of course, for the addition of functional groups to the pore surfaces.

A relative measure of the amount of functionality that has been imparted to the surface of the porous article may be obtained by using any of x-ray photoelectron spectroscopy (XPS), infrared spectroscopy or chemical analysis. The chemical reactivity of the surface, after remote gas-phase radical treatment as described herein, is also a very useful descriptor of the surface-functionalized articles.

To some extent, the reactivity of the surface is related to the amount of surface area: assuming uniform functionalization, the greater the surface area, the greater the amount of functional groups per gram of sample. For a porous article having a surface area of about 2,000 cm² per gram, functionalization according to the remote gas-phase radical treatment process described herein can provide about 0.01 to 1.0 μmoles of reactive functional group per gram of sample. Typical functionalization levels are 0.1 to 0.5 μmoles of reactive functional group per gram of sample, for a sample having a surface area of 2,000 cm² per gram as measured by Krypton gas adsorption measurements. This corresponds to reactive surface functionality on the order of 10⁻¹² to 10⁻¹⁰ moles/cm².

The amount of reactive surface functionality on a functionalized article will depend on the conditions under which the chemical reactivity is measured. Some swelling of the porous article will occur if the article is placed into an appropriate solvent. The swollen surface will tend to be more reactive with chemical reagents because more of the initially introduced functional groups will be accessible to the chemical reagents.

The chemical reactivity of a modified surface can be measured by many techniques. For example, the amount of reactive amino groups present on an aminated specimen may be determined chemically according to Allul, R., *DNA Probes*, Keller, H.G. et al., Eds. Macmillan, New York (1993). Thus, the aminated specimen is treated with 3-O-(4-nitrophenylsuccinylated)-5'-O-DMT-deoxyribonucleoside, followed by blockage of unreacted amines with pyridine/acetic anhydride/N-methyl imidazole (8:1:1, v:v:v). The amount of bound deoxyribonucleoside is determined by absorbance at 498 nm after treatment with 70% aqueous perchloric acid, toluenesulfonic acid in acetonitrile, commercial deblock preparations, or the like, to release the DMT group from the support.

By selecting the appropriate source gas, a hydrophobic precursor porous polymer article attains a substantially uniform layer of hydroxyl or amino groups. The surface functionality is spread across the entire surface of the article, which includes the exterior surface and the pore surface. Complete surface functionalization can be detected and distinguished from partial surface functionalization by wicking experiments with water, at least when the untreated surface is hydrophobic.

When oxygen is used as the source gas, the surface-functionalized porous article may have hydroxyl, carbonyl and carboxyl groups at the surface. Preferably, hydroxyl groups are the predominate functional group at the article's surface. If the surface content of carbonyl and/or carboxyl groups is undesirably high, such a functionalized polymer may be treated with a reducing agent, e.g., lithium aluminum hydride or sodium borohydride, to reduce all or essentially all of the carbonyl and/or carboxyl groups to hydroxyl groups.

Thus, subsequent to being exposed to the discharge, the surface-functionalized porous article having carbonyl and carboxylic acid groups (possibly in addition to hydroxyl groups) may be reacted with a reducing agent, so that hydroxyl groups are the predominant functional group bonded to the polymers which form the surface of the article. In addition, or alternatively, chemical agents that react with and cap (neutralize, eliminate) surface free radical sites and/or peroxides may be contacted with the surface-functionalized porous article. Such chemical agents include ammonia, dimethyl sulfide and other gases known in the art to cap/react with/neutralize free radical or peroxides on a polymer surface. Hindered amine light stabilizers, such as the Irganox™ products (e.g., Irganox™ 1076 and 1010) sold by Ciba-Geigy (Tarrytown, New York) and Cyanox™ 2246 from American Cyanamid (Wayne, NJ), as well as antioxidants such as 2,6-di-*tert*-butyl-4-methylphenol (BHT) and Nonox™ CI from

Imperial Chemical Industries, Great Britain, may also be used to cap these surface radicals or peroxides.

By use of ammonia or nitrogen/hydrogen source gas, the surface-functionalized porous article will have surface amino groups. A porous article having surface amino groups according to the invention may also be prepared from oxygen source gas, if the thus-formed hydroxyl-coated porous article is subsequently chemically treated to convert hydroxyl groups to amino groups. An effective chemical treatment may be accomplished by halogenation of the oxidized specimen to convert hydroxyl groups to halogen groups, followed by converting the halogen groups to amino groups, by any of known chemistry. Suitable halogenation chemistry includes treating the solid support with hydrochloric acid and zinc chloride at elevated temperature to provide chloride substitution, or phosphorous tribromide to provide bromide substitution. The Gabriel synthesis or Woff-Kishner chemistry may be used to convert the halogenated specimen to an aminated specimen.

Alternatively, the hydroxyl groups may be reacted with trifluoroethanesulfonyl chloride (Tresyl Cl) followed by ammonia according to Nilsson, K. et al., *Methods in Enzymol.* 135:65-67, 1987. Alternative approaches to converting hydroxylated specimens to aminated specimens may be found in Klein, E., *Affinity Membranes. Their Chemistry and Performance in Adsorptive Separation Processes*, Chapter 4, "Binding Chemistries," John Wiley and Sons, New York, 1991.

Subsequent to reaction with the flowing discharge radicals, the solid support may be briefly treated with an antioxidant, radical chain terminator (such as an alkyl thioether), or other stabilizer, so as to eliminate any residual organic peroxides or free radical sites. Reduction or other chemical modification of the support as described herein may then follow.

The surface-functionalized porous article preferably has the following properties. It is inert in that it will not degrade upon contact with chemicals to which it is exposed when it is used as a solid-phase support for a synthesis procedure. It should be "sturdy" in that it maintains its integrity during use. Thus, the article should not break into pieces if, for example, it is placed into a solution with a rotating mechanical stirrer. Also, if placed into a tall column, the lower portion of the article should not become crushed and perhaps plug a screen that holds up the article (in instances where a screen is used to support the article).

Other properties are also desired. The article should have a high surface area to volume ratio. In other words, for a fixed volume of article, it is desired to maximize the surface area over which the functional groups may be added according to

the present invention. The surface of the article should contain desirable "chemical handles" to which molecular fragments may be bonded, and should not contain any undesirable chemical groups which may interfere with the utility of the article.

D. AMPLIFICATION CHEMISTRY

5 Surface-functionalized porous articles as defined herein have surface oxygen or nitrogen-based chemical handles. In order to increase the number of reactive oxygen or nitrogen atoms, the surface-functionalized porous article may be subjected to amplification chemistry. As used herein, amplification chemistry refers to one or more reactions wherein a surface oxygen or nitrogen atom is reacted with a polyfunctional
10 chemical, so that a plurality of reactive functional groups are bonded through the surface oxygen or nitrogen atom to the porous article matrix. For example, a supramolecular structure may be covalently attached to the surface oxygen or nitrogen atoms, to produce a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, or a fractal polymer phase, where these supramolecular structures
15 contain a number of reactive functional groups that is in excess of the number of oxygen or nitrogen atoms that are used to join the supramolecular structure to the surface of the bulk matrix. One consequence of amplification chemistry is that a spacer moiety is positioned intermediate the surface oxygen or nitrogen atom and the functional group which is used to begin the solid phase synthesis. In addition, a single
20 chemical handle on the solid support is "amplified" into a plurality of chemical handles. Some exemplary amplification chemistry is described below.

 In order to amplify the hydroxyl and/or amino groups, these groups are reacted with multi-functional reagents. One such amplification approach is to graft
25 polymerize vinyl monomers onto the surface of the support. Such surface graft polymerization can be achieved either by immobilization of a vinyl monomer or polymerization initiator on the functionalized solid support, then immersing the radical-containing support into a solution of an appropriate vinyl monomer and subjecting the solution to polymerizing conditions by, for example, elevating the temperature.

 For example, a solid support containing surface amine groups may be
30 used to immobilize an AIBN-type initiator (e.g., 4,4'-azobis(4-cyanovaleric acid)) using carbonyldiimidazole coupling methods (Klein, E., 1991) or by prior conversion of the valeric acid functions in the initiator to acid chlorides at low temperature followed by low temperature coupling to the aminated MSPS. As used herein, the term "MSPS" refers to a solid support which has functionality that renders it suitable for use in at least
35 one of biomolecule synthesis, chromatography or diagnostics. The initial or derivatized

solid support can then be recovered, washed, and placed in a solution of appropriate vinyl monomers such as deinhibited vinyl acetate/acrylamide or vinyl pyrrolidone to produce a surface grafted polymer phase.

5 The grafting of polymers onto a solid support is illustrated with specific monomers in Figures 3A and 3B. Thus, the amine groups on a solid support may be reacted with maleic anhydride, to thereby form the corresponding maleimide. The maleimide group is then activated by reaction with a free-radical initiator, *e.g.*, AIBN, which introduces a secondary butyronitrile group as well as a free radical site. This is followed by reaction with acrylamide or acrylonitrile in an organic solvent such as acetone or under aqueous conditions, such that the level of free radical polymerization can be controlled stoichiometrically. These reactions are preferably conducted under an inert atmosphere, and monomer consumption (residual acrylamide or acrylonitrile) is monitored by gas chromatography. The resulting polymer chains are then reacted with AIBN or other free-radical scavenger to inactivate residual free radicals. The resulting maleimide groups, along with the nitriles or amide groups present on the polymer chains, are reduced to produce tertiary and primary amines, respectively. Suitable reducing agents include lithium aluminum hydride, sodium borohydride diborane and the like. In this way, a single amino group bound directly to the solid support has been elaborated to many amino groups joined to a polymer chain that extends from the single amino group.

20 An alternative approach to amplification is to conduct the free-radical polymerization described above in the presence of (meth)acrylic acid or (meth)acrylate ester, which provides a polymer chain having pendant carboxyl groups that may be reduced to pendant hydroxyl groups. Such an amplified solid support is particularly useful in PNA and peptide synthesis.

25 An alternative approach is to copolymerize either of the amine-precursor monomers (*e.g.*, acrylamide or acrylonitriles) or the hydroxyl-precursor monomers (*e.g.*, vinyl acetate) with a monomer which will not yield either reactive amine or hydroxyl groups. In this way, the reactive groups on the amplified solid support are spaced apart, which can lead to improved performance. An exemplary "inert" monomer is vinyl pyrrolidone, which upon reduction will provide a tertiary amine. Other "inert" monomers include styrene and *p*-aminoethylstyrene.

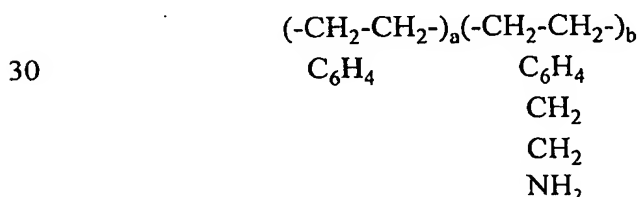
30 While reactive group amplification can be accomplished by free-radical polymerization from the initial reactive group directly bonded to the solid support, other mechanisms of polymerization may alternatively be employed. Such alternative mechanisms include anionic and cationic polymerization.

An alternative approach to reactive group amplification is to bond a pre-formed polymer to the initial reactive group, where the pre-formed polymer has a plurality of reactive functional groups (or masked precursors thereof). For example, amino groups on the solid support might be joined to a polyamine through glutaraldehyde. Cyanuric chloride bridging of amine, hydroxyls or the like may also be employed.

Another preferred multifunctional supramolecular structure is prepared by attaching a dendrimer to the solid support. Preferred dendrimers are Starburst™ dendrimers, hyperbranched dendrimers and fractal-polymer phases. Dendrimers provide stationary phases with high degrees of surface functionalization while being stable against surface restructuring with polar group involution that may be seen in some prior art plasma-treated polymers having extensive surface crosslinking. The dendrimers not only provide a high degree of functionality, but they also allow for a controlled microporosity to be placed over the surface of the solid support. The dendrimer may be pre-formed and then added to the solid support, or may be grown on the solid support by addition of monomers.

Dendrimers may be combined with other linker groups to impart a very high degree of functionality to the surface of a solid support. For example, amino-terminated 4th generation Starburst dendrimers may be reacted with R-NH-(N₃C₃Cl₂) or R-O-(N₃C₃Cl₂) to produce solid supports having up to 200 micromoles of amino function in 20 grams of resin (prepared from, e.g., T³ Porex filter).

The following calculations demonstrate how amplification can dramatically increase the number of reactive sites present on a solid support. When amplification is accomplished by converting an initial amine group to a maleimide, then forming a copolymer from the maleimide group using free radical polymerization and styrene and para-aminoethylstyrene as the co-monomers, the resulting copolymer has an average structure of:



For a 1:1 copolymer where a=b, the molecular weight of the average repeat unit is 126.5. In one embodiment of the invention, utilizing such a copolymer for the addition of 5000 amino groups per molecule of extended polymer, a length of 10000

monomers per chain is used. The molecular weight of this polymer is 1.26×10^6 Daltons. The space that such a molecule occupies is defined by the Flory Radius, R_f , of the polymer and also by the radius of gyration R_g of the statistical coil (with excluded volume effects). R_f is the extension of the polymer in a good solvent while R_g is obtained in a theta solvent at theta temperature. For a 10000 repeat molecule where one C-C bond length is 1.5×10^{-8} cm and 10^4 microns = 1 cm, then $R_f \times 10^4 = 10000^{(3/5)} \times 0.7 \times 1.5 \times 10^{-8} = 0.027$ microns, and for R_g where the C factor for a polymethylene chain = 6.7: $r_o \times 10^4 = (6.7 \times 1000 \times 0.7 \times (1.5 \times 10^{-8})^2)^{(1/2)} = 0.027$ microns, $R_g \times 10^4 = (r_o^2/6)^{(1/2)} = 0.011$ microns.

In this embodiment of the invention, such a polymer is built by extension or attachment, onto the internal bead walls of a sintered polymer filter, and, for example, spaced 0.019 microns apart on the internal surface area. For example, using a 30 micron pore size PorexTM with $0.113 \text{ m}^2/\text{gr.}$ of filter material, the number of polymer molecules = $0.113 / (19 \times 10^{-3} \times 10^{-6})^2 = 3.13 \times 10^{14}$ molecules/gr. For % weight per volume of polymer in the surface layer: $0.113 \times 10^4 \times 0.027 \times 10^{-4} = .003$ cc of volume in the surface layer, and $3.13 \times 10^{14} \times 1.265 \times 10^6 \times 1.650 \times 10^{-24} = 6.533 \times 10^{-4}$ gr. $6.533 \times 10^{-4} / (.003) = 0.217 = 21.7\%$ wt/volume in the surface area, comparable to the density of polyacrylamide gels used in protein and oligonucleotide electrophoresis.

The synthesis capacity in micromoles/gr. of solid support in this embodiment of the invention is: $(3.13 \times 10^{14} / (6.02 \times 10^{23} \times 10^{-6})) \times 5000 = 2.6$ Micromoles/gr. of 30 micron pore size material (e.g., PorexTM) $(3.13 \times 10^{14} / (6.02 \times 10^{23} \times 10^{-6})) \times 5000 \times 1.7 = 4.2$ Micromoles/gr of 7 micron pore size material (e.g., PorexTM).

It should be noted that these calculations estimate a minimum capacity based on simple geometric surface areas of the porous support as estimated by mercury porosimetry, and ignore the additional molecular area resulting from the supramolecular structure, especially in solvents that swell the surface phase. In both cases, the gel layer is only 0.027 microns thick prior to loading for synthesis. Increased capacity is obtained by making the gel layer thicker. The upper limit in thickness is when the loaded gel layer is 1/3rd the nominal pore diameter. The concentration of the loaded gel layer is also kept low enough for efficient mass transfer and chemical reaction.

The polymer chains are separated by 0.02 microns, while the length of a 60 mer oligonucleotide (which exceeds the current practical upper limit of oligonucleotide synthesis) = 0.02 microns, giving plenty of room for synthesis in these embodiments of the invention.

Functionalized solid supports according to the invention are commercially attractive materials for solid phase synthesis, chromatography, solid

phase medical assays and other uses because of their low cost of manufacture and their potential for high capacity and speed, as well as scalability. Capacity is a critical feature for the commercial utility of the supports; in general, the higher the capacity the better. Speed is also a key commercial issue, particularly the providing of quick
5 production cycles for oligonucleotide synthesis. A high flow rate, for example, is critical to oligonucleotide yield and quality, because low chemical residence times are necessary to prevent significant DNA degradation during the synthesis cycles.

The key issue is the resolution of the conflict between capacity and speed. When the number of functional groups is too low, capacity suffers. Too high a
10 polymer loading in the interstitial spaces of the porous supports can negatively impact flow rate. Thus, while both capacity and speed are critical to commercial success, a balance is necessary to achieve product viability.

The present invention as outlined above provides a chemistry and physical characteristics of design that enable resolutions of this conflict in a manner
15 unavailable in non-porous beaded or membrane configurations. This is because a functionalized solid support made from sintered polymeric particles has a fixed interstitial volume available for accommodation of synthesis or other solid phase processes. Furthermore, this volume is greater than a packed bed of beads and does not compress with use but is a part of a rigid, fixed structure, regardless of scale. The
20 sintered, three-dimensional bed has greater interstitial volume than a two-dimensional membrane. Accordingly, a preferred precursor porous article is sintered polymeric particles.

For example, one embodiment of the invention provides for direct treatment of sintered polymer beads with flowing discharge amine radicals. The
25 number of available $\text{-CH}_2\text{-}$ groups available on the interstitial surfaces for modification can be calculated from the known length of $\text{CH}_2\text{-CH}_2$ bonds, which are 1.5×10^{-8} cm (1.5×10^{-4} microns). For a 100×100 CH_2 planar area of polyethylene (10,000 CH_2 's), and given the known zigzag array of carbons (Gowariker et al., 1986), the length will be $0.7 \times 100 \times 1.5 \times 10^{-4}$ microns and the area = 1.1025×10^{-4} microns². For a 30 micron
30 pore size sintered polymer substrate (e.g., PorexTM), the interstitial surface area is $0.113 \text{ m}^2/\text{gr.} = 1.13 \times 10^{11}$ microns²/gr. The number of CH_2 groups per gram of PorexTM may be calculated by $10,000 \times 1.13 \times 10^{11} / 1.1025 \times 10^{-4} = 1.025 \times 10^{19}$ CH_2 's per gram. Thus, $1.025 \times 10^{19} / 6.023 \times 10^{17}$ molecules/micromole = 17 micromoles per gram of available CH_2 on the interstitial surface. For a smaller porosity substrate, e.g.,
35 5-7 microns, with 10 times the interstitial surface area, there is 170 micromoles of methylene groups per gram available for modification. Thus, with 20% amination of

interstitial surface methylene groups of polyethylene, direct use of the surface modifications, without amplification, can provide as much as 3.4 to 34 micromoles of functional groups per gram of sintered polymer substrate (for 30 and 5-7 micron pore size, respectively).

5 In the area of synthesis supports, and in order to have a 1 micromole capacity support, 60 to 600 microliters of bed volume will be required (for 30 and 5-7 micron pore size, respectively). Furthermore, additional capacity can be gained by use of even smaller pore size, longer or higher energy flowing discharge treatments to induce pitting onto the surface of the sintered beads, increasing the interstitial surface
10 area, and/or by polymer extension or grafting with polyfunctional compounds, as described above.

E. MODIFIED POROUS ARTICLES

 According to the methodology described herein, the invention provides modified porous article wherein non-native surface-bonded oxygen and/or nitrogen
15 atoms are also bonded to one or more functional groups to form new molecular and supramolecular structures on the surface of the precursor article. Such modified porous articles may be generally represented by the structure I, wherein "SS" represents the solid support, "X" represents an oxygen or nitrogen atom, and "f" represents a
20 functional group.



 In a preferred embodiment, an amplification group L (a "spacer group") as described above, is interposed between the surface oxygen and/or nitrogen atom X and the
25 functional group f, such that a plurality ("m") of f groups are bonded to the amplification group L, as shown in Structure II.



30 F. CHEMICAL MODIFICATION OF PLASMA-TREATED POROUS ARTICLES HAVING AMINO FUNCTIONALITIES

 Following successful amination using the plasma treatment methods described above, lipophilic materials like polystyrene, polyethylene and polypropylene

can be surface modified through the newly introduced amino functionality. Macroporous, non-swelling polystyrene beads can also be modified. A vast array of chemistry can be carried out directly on the amino function introduced by plasma treatment, but in many examples, it is advantageous to introduce a spacer between the surface of the bulk polymer and the reactive functional group. The spacer serves to eliminate or reduce steric interactions due to the bulk polymer's surface and to solubilize the reactive functional group thereby facilitating chemical reactivity in the solution phase. This document describes methods involving synthetic chemistry that will increase the capacity and performance of macroporous, non-swelling polystyrene particles.

(1) *ATTACHMENT OF LINEAR POLY(ETHYLENE GLYCOL) (PEG) DERIVATIVES TO PLASMA-TREATED NON-SWELLING MACROPOROUS POLYSTYRENE PARTICLES*

If a linear linker is to be coupled to the plasma-treated macroporous material, the number of reactive functional groups is not amplified, nonetheless, one attains the necessary properties of the spacer in order to increase the chemical reactivity of the functional group. An important approach for coupling a spacer molecule to the plasma-modified surface is through a reactive intermediate. As shown in Figure 6, such an intermediate is the reactive imidazolyl urea (2), which can be easily generated by treating the plasma-treated material (1) with carbonyldiimidazole in a suitable organic solvent such as dioxane. The activated intermediate can then be attacked by a nucleophile, such as a linear, bifunctional poly(ethylene glycol) (PEG) linker in an appropriate organic solvent such as acetonitrile. The result of such a conversion is a solid material that has a highly stable and swellable linker arm with a terminal amino functionality that is amenable to synthetic modification (3). The stable urea linkage between the PEG and the plasma-treated material is essential so that the PEGylated part of the structure is not cleaved from the solid support and contaminates the solution surrounding the solid phase material. The importance of the swelling characteristics of the PEG is to ensure that the reactive amino group at the end of the linker has a solution-like environment as opposed to a solid-like environment for chemical reactivity. The amino group at the end of the linear linker can then be modified depending on the application; if it is loaded with a suitably protected deoxynucleoside succinate, DNA synthesis is possible on an automated instrument. If the amino group is quaternized, anion exchange chromatography is possible. In lieu of a thoroughly characterized (and costly) PEG, a wide array of linear (and inexpensive) PEGs

diamines, commonly known as Jeffamines®, can be used in conjunction with the imidazolyl urea intermediate (2). The use of excess Jeffamines® ensures that the increase in cross-linking at any level is insignificant.

The type of PEG linker that is used is fundamentally important. If a well characterized, activated PEG that has a protecting group on one end, such as *t*-Boc-NH-PEG-NHS, is used, additional cross-linking and side reactions can be minimized and an efficient transformation can be attained. In this type of approach, the amino functionality on the plasma-treated material reacts smoothly with the activated NHS end of the PEG molecule to form an amide linkage. The *t*-Boc group protects the amino functionality at the other end of the PEG from chemical reactivity. Once the linkage is formed, the *t*-Boc protecting group is removed with trifluoroacetic acid (TFA) to reveal a terminal amino group. As shown in Figure 6, the resulting structure (4) is similar to that described above (3), except that the linkage between the modified surface and the PEG is an amide linkage. This new material (4) can also be further modified for the appropriate application, DNA synthesis, anion exchange chromatography, or for attaching moieties, such as antibodies, useful in medical assays.

Examples 10-14 illustrate methods for attaching linear PEG derivatives to plasma-treated non-swelling porous polystyrene particles.

20 (2) BUILDING DENDRITIC MOLECULES ON PLASMA-TREATED MACROPOROUS MATERIALS

In certain cases, it may be necessary to increase the number of reactive functionalities on an plasma-treated material. An approach for such amplification is the stepwise addition of molecules that yield dendritic structures. Dendrimers are tree-like macromolecular polymers that contain a repeating structural unit. Dendrimers can be constructed by the repetitive cycle of adding acrylonitrile and the reduction of the resulting cyano groups with DIBAL (see, for example, Moors, R. and Vogtle, F., *Chem. Ber.* 126:2133, 1993). Thus, when the plasma-treated material (1) is treated with acrylonitrile in glacial acetic acid for 24 hours, a bis(cyanoethylamine) results. See Figure 7. The cyano groups of this intermediate are then reduced with diisobutylaluminum hydride in THF, regenerating amino functionalities. Each 2-step cycle doubles the number of amino functionalities. Hence, if the starting plasma-treated material, with *n* amino groups, can undergo one dendritic building cycle, a structure with 2*n* amino functional groups results (5). After two complete cycles of dendrimer buildup, a structure with 4*n* amino functional groups is generated (6). The addition/reduction cycle can be repeated until the desired level of amplification is

obtained. Once the amino groups have been amplified to the appropriate level, synthetic modification for the appropriate application can be performed. Thus, loading the amplified material with a deoxynucleoside succinate will generate a structure (7) that will enable DNA synthesis. Alternatively, quaternization of the amino groups with
5 iodomethane and tri-*n*-butylamine followed by hydrolysis with silver oxide yields a material suitable for anion exchange chromatography (8). The general idea behind building dendritic structures on the surfaces of plasma-treated materials is to increase the capacity of the resulting material. Accordingly, such material is useful for solid phase biochemical synthesis, chromatography, or medical assays. Regardless of the
10 application, one will find that these materials (those with structures similar to 7 and 8) will have a larger capacity than those described in the previous section. Example 15 illustrates a method for producing dendritic molecules on plasma-treated porous materials.

15 (3) *COUPLING STARBURST PAMAM DENDRIMERS TO PLASMA-TREATED MACROPOROUS MATERIALS*

An alternative approach to amplification of the functional groups on an plasma-treated material is to introduce pre-formed dendritic molecules (Starburst PAMAM dendrimers). Such an approach involves the reaction of the reactive
20 imidazolyl urea intermediate (2) with the dendrimer, affording a material with a stable urea linkage between the solid material and the dendrimer (9). See Figure 8. This approach is highly prone to degrees of cross-linking since the pre-formed dendrimers have nucleophilic amino groups within the same branch of the molecule, which can attack other proximal imidazolyl urea functionalities. The degree of cross-linking can
25 be lowered by using excess Starburst PAMAM dendrimer, but the potential for intrabead cross-linking will always be present to some degree by this approach. The level of amplification is controlled by the dendrimer that is used. Starburst PAMAM dendrimers are widely available and may have as few as two surface amino groups and as many as 64 surface amino groups. A material with an amplified number of amino
30 groups (9) can then be modified for an appropriate application, such as solid phase biochemical synthesis, chromatography, or solid phase medical assays. Thus, loading the amplified material with a deoxynucleoside succinate will generate a structure (10) that will enable DNA synthesis. Alternatively, quaternization of the amino groups with iodomethane and tri-*n*-butylamine followed by hydrolysis with silver oxide yields a
35 material suitable for anion exchange chromatography (12).

Examples 16-19 illustrate methods for coupling Starburst PAMAM dendrimers to plasma-treated porous materials.

(4) *COUPLING STAR PEGS TO PLASMA-TREATED MACROPOROUS MATERIALS*

5 Star PEGs are multi-branched versions of poly(ethylene glycol) and can have up to 100 branches (13). See Figure 9. These Star PEGs can be used in conjunction with the versatile, activated imidazolyl urea intermediate (2) to amplify the available number of functionalities. The amplified functionalities in this case turn out to be hydroxyl groups obtained by introducing the Star PEG molecules through a short
10 carbonyl spacer. Once the attachment of the Star PEG molecule has been accomplished (14), the object becomes the transformation of the hydroxyl groups into the more nucleophilic amino groups (17). The general idea for this transformation is to convert the hydroxyl groups to good leaving groups (chloro or methanesulfonate) that can then be displaced by a good nucleophile (ammonia, or any diamine such as
15 ethylenediamine). One possible path for this transformation involves the conversion of the hydroxyl groups into chloro groups by using oxalyl chloride. This transformation occurs under mild conditions and affords material having the general structure of 15. The chloro substituents on (15) can then undergo a smooth displacement with any amine. While we illustrate the displacement with ammonia to afford material 17, we
20 have found that these reactions work very well with various diamines such as ethylenediamine and the Jeffamines®. An alternate approach for the conversion of hydroxyl groups into amino groups involves the generation of methyl sulfonates (16), which are ideal leaving groups in the presence of an amine. The displacement of methane sulfonates with amines (ammonia, primary amines, or various diamines such as
25 ethylenediamine or the Jeffamines®) is an efficient reaction under mild conditions. Once the conversion of hydroxyl to amino (14 to 17) is complete, the appropriate modification for the pertinent application can be achieved. Such material can be used for solid phase biochemical synthesis, chromatography, or solid phase medical assays. Thus, loading the aminated support with a suitable deoxynucleoside succinate will
30 result in a DNA synthesis support (18) and quaternization with iodomethane (19) followed by hydrolysis with silver oxide will ultimately result in an anion exchange resin (20). See Figure 10. Examples 20-26 provide illustrative methods for coupling STAR PEGs to plasma-treated porous materials.

2. Use of Porous Articles for Solid Phase Biochemical Synthesis

Clinical successes are driving needs for commercial scale manufacturing of biomolecule and organic compound therapeutics into reality. While current approaches to solid phase synthesis are reasonably satisfactory on a small scale in a laboratory environment, there is a great need for supports that allow solid phase processes to be readily scaled-up to the multi-kilogram scale and beyond in an economical manner. At the discovery end of the scale, solid phase approaches to micro scale combinatorial screening of synthetic DNA and analogs would be of even greater value if they could be quickly scaled-up to manufacturing.

A. PARTICULARIZED SUPPORTS FOR BIOCHEMICAL SYNTHESIS

The present invention provides porous articles particularly suited for the solid phase synthesis of biomolecules. The articles have functional groups through which solid phase synthesis may be initiated, yet do not suffer from a loss in mechanical properties that typically accompanies the introduction of such functional groups. Thus, the modified article (*i.e.*, the article that contains the functional groups through which solid phase synthesis may be initiated) has essentially the same mechanical properties as the precursor to the modified article (referred to herein as the precursor porous article).

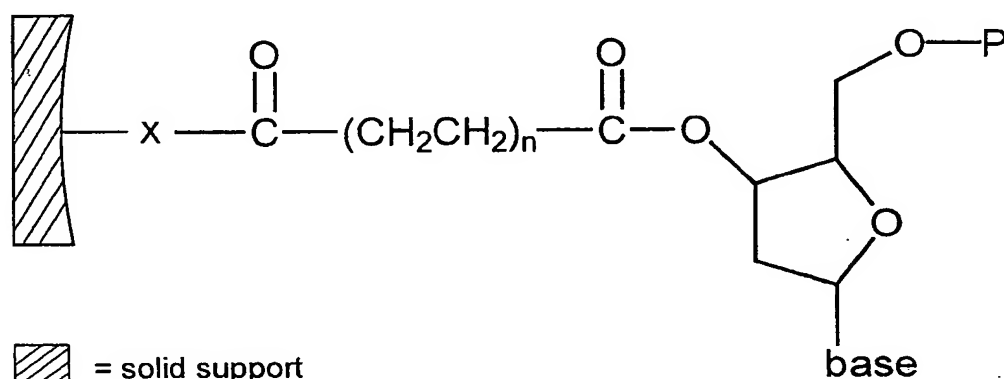
The modified porous articles of the invention (such as Structure I or II), for example, may function as a solid phase support for the synthesis of organic and biomolecules. Exemplary biomolecules that may be prepared with the solid phase support of the invention include oligonucleotide, polynucleotides and nucleic acids, (*e.g.*, DNA, RNA and PNA (peptide nucleic acids), in linear or cyclic form) as well as analogs thereof, peptides (including linear or cyclic oligopeptides, polypeptides and proteins) and peptide analogs including peptide mimics and mimetics, and oligosaccharides, polysaccharides and analogs thereof. Essentially any organic or biomolecule that can be synthesized on a solid support, may be synthesized using the modified porous articles of the present invention. These modified porous articles may be used in laboratory scale research as well as commercial production scale reactions.

Oligonucleotide synthesis technology which may be performed on the solid supports of the present invention encompasses natural DNA structures as well as many synthetic modifications, including phosphorothioates (Zon and Geiser, 1991), phosphoramidates (Froeler, 1986, and Dahl, B.H. et al. *Nucl. Acids Res.* 15:1729-1743 (1987), phosphoramidate chemistry;), peptide nucleic acids (PNA's, DeMesmaecker

et al., 1995), 3' amines, and their modified versions (Asseltine and Thuong, 1990, Vu et al., 1993, 1995), as well as other synthetic oligonucleotides (Matteucci and Caruthers, 1981; Gough et al., 1981; Sinha et al., 1984; Atkinson and Smith, 1984; McCollum and Andrus, 1991; Alul, 1993).

5 The solid supports as described herein may be used for pharmaceutical or other applications, such as PCR and analysis, in diagnostics or research. These solid phase supports may also be used for microscale combinatorial screening of synthetic DNA and analogs.

10 In one embodiment of the invention, a functionalized solid support which is particularly well suited for oligonucleotide synthesis has the following Structure III



Structure III

15

The synthesis supports of Structure III may be prepared from either hydroxylated or aminated resin, giving rise to supports wherein X is O or N, respectively. The hydroxylated or aminated support is then reacted with either succinyl chloride or oxalyl chloride, (n = 1 or 0, respectively, in Structure III). Next, the 5' hydroxyl group of a protected deoxynucleoside is reacted with the residual acyl chloride

20 hydroxyl group of a protected deoxynucleoside is reacted with the residual acyl chloride group of the succinyl or oxalyl chloride, to provide Structure III.

The solid phase supports of Structure III may be employed with standard techniques for conducting solid phase synthesis of oligonucleotides, as are well known in the art. This typically requires the preparation of four different solid supports, each

25 having either adenine, guanidine, cytosine or thymidine as the base B in Structure III. The appropriately functionalized solid support is selected in order to provide the desired

first base in an oligonucleotide sequence. Subsequent deoxynucleosides may be added to the support by standard phosphoramidite chemistry (Dahl et al., 1987). Such chemistry may be conducted in a syringe (Tanaka and Letsinger, 1982) or by an the automatic technique using any of a variety of commercially available instruments such as the Applied Biosystems 380B synthesizer (Brown and Brown, 1991). The success of DNA synthesis may likewise be determined by standard techniques. *See, e.g.,* Vu et al., 1995.

The modified porous articles have many advantages over presently used "particulate" nucleic acid synthesis support systems, *e.g.,* Controlled Pore Glass (CPG) (Matteucci and Caruthers, 1981, Gough et al., 1981), highly cross-linked polystyrene (McCollum and Andrus, 1991), polystyrene polyethylene glycol copolymer, *e.g.,* Tentagel™ (Bayer and Rapp, 1986), and polystyrene Primer Support™ (Pharmacia) resin which are currently used or proposed for use commercially in oligonucleotide synthesis.

In distinction from the supports of the present invention, the particulate porous resins of the prior art require agitation to assure complete equilibration at each step in the DNA synthesis cycles. This is necessary for successful coupling, complete washings, and to minimize the production of incorrect "failure" sequences (Gough et al., 1981). In the smaller scale automated DNA synthesizers, *e.g.,* Applied Biosystems' 380B and PerSeptive Biosystems' Expedite™, agitation is accomplished by reverse flow through incompletely filled columns with filters at both ends. In the larger scale machines, for example, the Applied Biosystems 390Z and PerSeptive Biosystems 8800, agitation is accomplished by physical shaking or sparging with inert gas. Such a need for agitation introduces complication and expense to the oligonucleotide process. Furthermore, as batches become larger during scale-up, equilibration becomes more problematical, due to support particle fragmentation, water entrainment, and incomplete agitation (Alul, 1993). With the synthesis support matrix within the interstices of a three-dimensional sintered porous polymer filter according to the invention, complete equilibration can be obtained in a simple, low-pressure, high-rate, high-load, flow-through filter reactor configuration, without the need for agitation, regardless of the scale of synthesis.

Automation of oligonucleotide synthesis using prior art supports must be designed to deal with particles that escape from the solid column or bed. This is the case with particulate resins such as controlled pore glass that contain fines which have to be screened out to prevent clogging up of the filters that retain the particulates. Clogged beds lead to reduced flow and poor product quality. Some of the prior art supports suffer from poor mechanical stability. Thus, particulates may break free of the

resin beds, causing clogging of lines in synthesizers. Such clogging is a major cause of maintenance and production downtime. The presence of such fines in the particulate supports has an important effect on support quality by producing variability in quantitative loading and support stratification.

5 Synthesis procedures using prior art supports are not easily (and in some cases, are not possibly) scaled up when the need for production size batches arises. While particulate non-porous resins are being used in a flow-through mode in some higher scale applications (Glasser, 1996), the low loading of the non-porous polystyrene Primer Support[™] (Pharmacia) requires large reactors. Due to the particulate nature of
10 that support, scale-up substantially lowers flow rates due to column packing, causing limitations on scale potential, unduly prolonging cycle times, raising production costs and negatively effecting quality and yield.

 The invention also provides advantages over membrane (non-particulate) synthesis supports (*e.g.*, MemSyn[™], PerSeptive Biosystems and Matson et al., 1994),
15 which have much less interstitial volume and loading, and markedly limiting synthesis capacity, compared to the solid supports of the present invention. Loading capacity is a particular problem for the other commonly used flow-through (non-particulate) synthesis support, a membrane support, MemSyn[™] (PerSeptive Biosystems). This has minimal loading capacity due to its small pore size. Also, the essentially two-
20 dimensional structure of a membrane inherently has much less interstitial volume to accommodate synthesis than does a three-dimensional structure. Membrane supports also have poor flow characteristics, so they cannot be stacked to increase capacity as the chemical residence times during synthesis cycles are crucial for product quality. Consequently, membrane supports are only suitable for the lowest scales of synthesis.

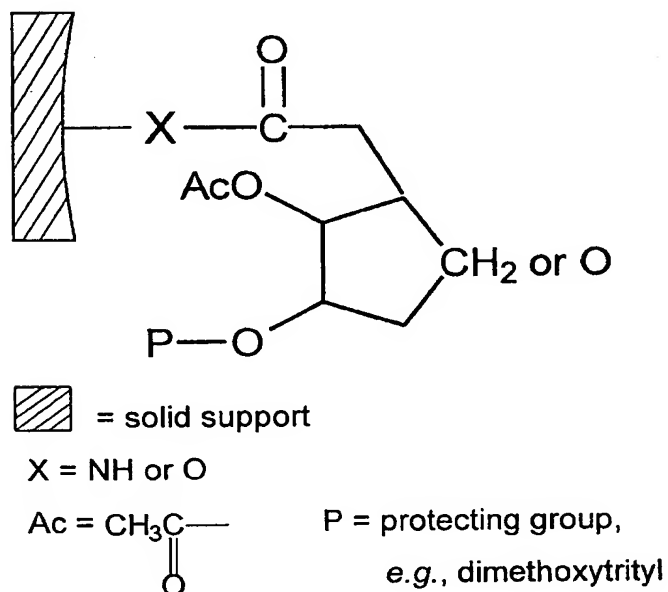
25 The polypropylene membrane and non-porous synthesis supports derived from direct plasma amine radical treatment (Matson et al., 1994) have even lower loading than the commercial membrane supports (*e.g.*, MemSyn[™]). Direct plasma treatment does not penetrate the interstices of membranes or any other porous polymers and can only be used sparingly due to polymer degradation.

30 The functionalized solid supports of the present invention are readily used in standard solid-phase synthesis protocols. This is the case whether the functionalized solid support is used for the low scales of combinatorial drug discovery, the commercial high throughput low scales to supply materials for PCR and research, or the commercial large scale manufacture of pharmaceuticals, etc. The solid phase
35 synthesis supports of the present invention significantly decrease product and process development time as well as development, capital, and manufacturing costs.

As stated above, the solid phase synthesis supports of the present invention may be used as synthesis supports for molecules other than oligonucleotides. For instance, they may be used in peptide and PNA synthesis, in lieu of, *e.g.*, non-swelling Poros[™] particulate supports which consist of a copolymer of polystyrene/divinylbenzene (9/1) and polyethylene glycol (PerSeptive Biosystems), and which suffer from limited loading and poor flow characteristics.

B. UNIVERSAL SUPPORTS

The invention also provides for so-called "universal supports" which have the following Structure IV.



Structure IV

In Structure IV, the solid support may be any insoluble organic or inorganic material which is inert to the reaction conditions employed for the solid phase synthesis. Suitable solid supports for solid-phase oligonucleotide synthesis are known in the art, and include controlled pore glass beads, polystyrene beads, polystyrene/divinylbenzene beads, polystyrene/polyethylene glycol beads (*e.g.*, Tentagel[™]), polymeric membranes (*e.g.*, MemSyn[™]) and polymeric films, to name a few. Of course, the solid support may also be sintered polymer beads, such as the functionalized porous articles of the present invention.

Alternatively, the solid support in the above structure may be prepared by direct plasma treatment or corona discharge of a porous precursor article, which can generate atomic oxygen and amine radicals that will react with a solid support. Chemical oxidation of a solid support using dichromate or permanganate salts, peroxides or strong oxidizing acids may be employed to introduce the chemical handles which are elaborated to provide the universal support shown above. As another alternative, a precursor solid support may be subjected to hydrogen abstraction by, *e.g.*, photoinitiation using benzophenone, tetramethoxycarbonyl benzophenone or the like, or atom transfer radical initiation (*see* Patten et al., 1996), or hydroxyl or amino radicals derived from chemical initiators such as titanous chloride-hydrogen peroxide or titanous chloride-hydroxylamine, in order to prepare an aminated specimen. The precursor to the specimen which has the "chemical handles" may be a fiber mat filter, a woven fabric filter, GorTex™ fabrics and filters, macroporous solids produced by staged templated suspension/emulsion polymerization processes, and perforated polymer sheets or films, to name a few.

Regardless of the exact structure of the solid support, or the manner in which it was prepared, universal supports of the present invention have, directly bonded to the solid support, either an ester (-O-C(=O)-) or amide (-NH-C(=O)-) group, which links the solid support to a functionalized 5-membered ring as shown in Structure IV.

The functionalized support of structure IV may be prepared by reacting a hydroxylated or aminated solid support with a carboxylic acid or reactive equivalent thereof, for example, 2-cyclopentene-1-acetic acid. Further treatment with cold dilute neutral potassium permanganate converts the cyclopentene to a *cis* vicinal dihydroxy cyclopentane derivative. This derivative is treated first with dimethoxytriphenylmethyl chloride (DMT-Cl) to protect the hydroxyl group furthest from the appended methylene group as the DMT ether, and then with acetic anhydride to protect the remaining hydroxyl group as the acetate.

The functionalized support of Structure IV is useful as a universal support for oligonucleotide synthesis. The term "universal" means that it can be used regardless of which base is desirably placed in the first position of an oligonucleotide to be synthesized. To achieve oligonucleotide synthesis, the Structure IV is treated with mild acid, which removes the DMT group and leaves a free hydroxyl group attached to the 5-membered ring. This free hydroxyl group may then be elaborated to an oligonucleotide by standard oligonucleotide chemistry. When it is time to cleave the oligonucleotide from the solid support, the phosphorous group attached through the

oxygen atom to the 5-membered ring will break, thus freeing the oligonucleotide from the solid support.

Prior art "universal" supports include the BioGenex Universal Support and the Clontech Rainbow™ Universal Support. In contrast to the prior art universal oligonucleotide supports, the support of the present invention does not require a
5 succinyl bridge between the support and the first attached oligonucleotide (as both the BioGenex and Clontech products do).

C. A POLYMER COLLOID PELLICULAR MONOLITH SOLID PHASE SYNTHESIS SUPPORT USEFUL FOR BIOCHEMICAL SYNTHESIS

10 Examples 27 and 28 detail methods for producing a polymer colloid pellicular solid synthesis support. Such a support can be used, for example, to synthesize DNA. Briefly, a stable polymer colloid dispersion consisting of polymer colloid particles having amine reactive functional groups reacts with the surface amine
15 functions of plasma-modified Porex X-4920 so that the polymer colloid particles are covalently coupled to the amino, plasma-treated pore surfaces throughout the porous matrix. The polymer colloid particles are coupled to the amino plasmid-treated material using only a limited number of the amine reactive groups near the surface of the particles. The vast majority of the reactive groups contained within the particles are still available for derivatization on swelling of the pore-surface coupled particles in a
20 suitable organic solvent.

D. DEVICES FOR SOLID PHASE SYNTHESIS

Another aspect of the invention is devices designed to capitalize on the advantages of the solid phase synthesis supports of the invention. One such device is
25 illustrated in Figure 4. Figure 4 shows a cassette comprising a cylindrically-shaped monolithic synthesis support of the invention, inserted into the barrel of a housing, where the housing has a length (l) and a diameter (d). The synthesis support may be a stack of discs, rather than being monolithic. However, because the synthesis support fits snugly within the housing, and is either monolithic or formed of a plurality of
30 stacked discs, there is no need for frits at either end of the reaction chamber: no particulates are either present within the f-MSPS or will break free therefrom. In order to provide a connection to tubes at either end of a reaction chamber, each end is tapered to a Luer configuration. This is especially convenient when the device will be fitted

into an automated oligonucleotide synthesizer, *e.g.*, PerSeptive Biosystem's Expedite™ DNA synthesizer.

The size (l/d) of the cassette, and the loading (concentration) of the functional group f on the solid phase synthesis support, is selected to accommodate any of the various commercial small scale synthesizers. For example, with an appropriate l/d and a loading for a capacity of 0.05, 0.2, 1, and 15 μ Moles, or above, a cassette is provided which can be used in the PerSeptive Biosystems Expedite™ oligonucleotide/PNA synthesizer, the Applied Biosystems Perkin Elmer 380B oligonucleotide synthesizer, or the like.

For larger scale solid phase synthesis, the device of Figure 5 is well-suited. Figure 5 illustrates a solid phase synthesis support of the invention fitted within a housing. The housing has two end pieces, through which solutions enter and exit, respectively. The housing also has side surfaces, to help retain solutions within the solid support. The end housing through which solutions enter preferably has a labyrinthine design to permit better distribution of flow across the reactor/separation chamber. The end and side housings may be made of plastic, *e.g.*, polyethylene, which can snap together to form a liquid-tight seal.

If desired, the entrance and exit ports of the end pieces can be configured in a manner complementary to that found in a commercial chemical synthesizer, *e.g.*, the PerSeptive Biosystems 8800 Oligonucleotide Synthesizer, a Pharmacia Biotech Oligo Pilot II DNA/RNA Synthesizer, or the like, which can accomplish commercial pilot-plant scale synthesis. Similarly, the entrance and exit ports can be configured in a manner complementary to fit commercial scale synthesizer, such as the Pharmacia Biotech OligoProcess DNA/RNA Synthesizer or the like. Likewise, the dimensions of the solid phase synthesis support, as well as the loading capacity thereof, can be tailored to accommodate the requirements of the commercial synthesizers.

The monolithic nature of a preferred solid phase synthesis support of the invention provides many advantages which are recognized in the parts of the device shown in Figure 5. For instance, there are no end frits, plungers or the like to retain the particulate material that is commonly employed in reaction and separation columns. Furthermore, the device makes no accommodation for the user to gravity pack a column, because the solid phase synthesis support is simply inserted into the housing. While the solid phase synthesis support is illustrated as a single piece, in fact it may be formed of more than one short piece, where the shorter pieces are stacked one on top of another to fill the housing.

The following U.S. Patents describe methods for modifying the surface of an organic polymer: U.S. Patent No. 5,369,012, U.S. Patent No. 5,332,551, U.S. Patent No. 5,215,790, and U.S. Patent No. 5,141,806.

5

3. Use of Porous Articles for Chromatography

In the liquid chromatography art it is known to provide solid support matrices (or "stationary phases") for the separation of mixtures of solutes in a solution (or "mobile phase") on the basis of physicochemical properties. Examples of such
10 physiochemical properties include molecular size, charge, hydrophathy (relative hydrophobicity or hydrophilicity), conformation and the like. Separations may also be achieved by taking advantage of differences in hydrodynamic properties between the solutes, including specific and non-specific interactions, for example affinity interactions, between the solutes and the stationary phase.

15

According to typical chromatographic practice, a liquid sample mixture containing multiple distinct molecular species dissolved in an appropriate solvent, and whose separation from one another into discrete components is sought, is applied to a suitable solid phase and permitted to flow through interstices in the solid phase. Additional mobile phase solvent is applied to the solid phase such that liquid flow
20 through the solid phase is effected and liquid effluent containing the separated molecular components of interest can be recovered. Depending on various factors, such as the physicochemical properties of the solute compounds to be separated, as well as the nature of the stationary phase and of the mobile phase, separation is accomplished by exploiting different molecular characteristics of each.

25

Because of the lability of many biologically important molecules, particularly under non-physiological conditions as might be necessary for a chromatographic separation, rapid purification procedures, to achieve either partial or full purification, is often highly desirable so that the molecules of interest are exposed to potentially destabilizing conditions as briefly as possible.

30

It is known in the art that chromatographic resolution, or separation of the distinct components in a sample, is a function of a column solid support's selectivity (relative retention of a particular sample component), its capacity factor (the ratio of sample mass in the stationary phase to that in the mobile phase) and its efficiency (resolving power expressed as "theoretical plates" and quantified based on the ratio of a
35 sample component's peak retention time to its band width). Because chromatographic separations depend upon the interaction of dissolved sample (a solute) with the solid

support of the stationary phase, the speed of a separation is limited by the rate of solute diffusion within the mobile phase.

Sample dispersion, also known as "bandspreading", which reflects decreased resolution, increases when flow rate is increased at the expense of opportunities for diffusive sample interaction with stationary phase. Bandspreading can also be controlled by various aspects of the solid support's geometry and its influences on sample interaction with the stationary phase. For example, accessibility of stationary phase surface area to a sample may depend upon suitable stationary phase pore size and on the diffusion constant of the sample, both of which are functions of the sample compound's molecular weight. Accordingly, it is known in the art how to conduct chromatographic separations by selecting solid supports and flow rates that permit necessary interactions between the sample and the stationary phase while reducing bandspreading.

However, deficiencies in the properties of solid supports that are known in the art preclude further minimizing the bandspreading of samples whose separation is sought. It is an aspect of the present invention to provide solid supports for chromatography that permit high resolution by overcoming such deficiencies.

Particulate solid supports packed into cylindrical columns for liquid chromatography are among the most well known and widely used solid supports. However, a number of the particulate solid supports in the prior art comprise particles of soft or compressible materials such as cross-linked cellulose, dextran, agarose or the like. These solid supports suffer from a number of limitations that compromise their effectiveness as useful chromatography packings.

One such limitation is due to the compressibility of a solid support. Compressible solid supports cannot be used at high flow rates because the pressure generated by a high flow rate causes the particles to collapse. Irregularities in the particulate solid support bed are produced by such compression, including the formation of channels within the bed that create uneven mobile phase flow paths in different regions of the column. Resolution is sacrificed when sample in the mobile phase is retained to varying degrees in different column microenvironments because such uneven mobile phase flow paths impair uniform sample interaction with the stationary phase.

Increasing the rigidity of chromatography solid supports to make them less compressible is accomplished by producing particles having an increased proportion of their volume occupied by solid stationary phase material, thereby correspondingly reducing particle porosity (the volume of each porous particle taken up

by mobile phase). Such reduction of porosity has the undesirable consequence of decreasing solvent-accessible stationary phase surface area per unit volume, with a resultant loss of resolving power in chromatographic separations. For the same reasons, nonporous particles fabricated from suitable stationary phase materials, or nonporous particles coated with surface layers of porous stationary phase materials, are imperfect alternatives.

Another problem with the solid supports known in the art is that they exhibit poor mechanical strength and tend to fragment and crumble into "fines." Fines are a significant problem because they can interfere with solvent flow, clog the chromatography supports, and reduce the resolving ability of chromatography columns. Removal of fines from solid supports is a difficult, time-consuming and costly process that compromises the overall usefulness of chromatography stationary phases.

Yet another problem with many currently used solid supports is related to the chemical composition of the supports. The chemical composition of many solid supports is such that they do not resist chemical attack or solubilization by any number of mobile phase components that are desirable for optimizing a chromatographic separation. For instance, certain solvents or pH extremes which may be highly desirable to achieve a certain separation are precluded due to the chemical sensitivity of the solid support.

It is an aspect of the present invention to provide porous solid chromatography supports that are not compressible, that do not degrade to form fines, and that are chemically resistant to a broad range of mobile phase compositions. These chromatography supports according to the invention further provide a high surface area to volume ratio, as is desirable in a stationary phase providing useful chromatographic resolution of samples.

Particulate stationary phases made of porous silica are popular chromatography solid supports. These particulate silica stationary phases possess some improved properties over the solid supports of the prior art described above, such as improved mechanical strength and reduced tendency to collapse. However, silica-based stationary phases suffer from numerous shortcomings that make them non-ideal for chromatography. For example, efficient packing of silica particles into chromatography columns is difficult. In addition, sample interaction with the stationary phase is still diffusion-driven, and silica particles having useful pore sizes require very high pressure drops to achieve adequate resolution. Such high pressures can cause compression of column beds even in silica-based chromatography supports, and this brittle material can

crumble into fines. Thus silica requires special care in fabrication, handling and packing into chromatography columns or other devices.

As with the other supports, increasing the pore size of silica-based chromatography media decreases overall mechanical strength, reduces sample capacity and compromises the resolution that can be attained. In addition, silica is not stable over a broad pH range, and this fact compromises its usefulness for numerous chromatography applications, particularly those employed for the chromatographic separation of biomolecules.

Protective chemical modifications of silica particles to improve their chemical resistance and/or to introduce desirable chemical functionalities to the particle surface, such as the process of "end-capping," are difficult to achieve quantitatively and further involve added risk of mechanical damage to the brittle particles during processing and handling. Other shortcomings of silica-based chromatography supports are known to those familiar with the art.

Synthetic polymer-based particulate solid supports for liquid chromatography are also known in the art. For example, such polymer-based solid supports may be produced by suspension polymerization. Such polymeric particles represent another approach to provide improved stationary phases. However, from the perspective of chromatography theory these particulate supports, which typically employ particle geometries similar to those used in the stationary phases described above, suffer from the same inherent limitations on capacity, speed and resolution as particulate stationary phases made of other materials. Also, it is difficult and expensive to obtain preparations of this type of particle having uniform particle size, an important consideration for producing a high resolution chromatography column.

Additionally, limitations on the chemical compatibilities of materials that may be suitable for the suspension polymerization process used to produce particulate synthetic polymeric stationary phases restrict the range of compositions that may be used. Therefore, it may be impossible to synthesize specific polymeric stationary phases having particularly desirable surface chemical properties that would be useful for certain chromatographic separations. This limitation may further restrict the range of stationary phase surface chemistries that can be produced as derivatives of particles prepared by suspension polymerization.

It is an aspect of the present invention to provide porous solid stationary phases for liquid chromatography that can be made of essentially any synthetic, semisynthetic or naturally occurring organic polymer having hydrogen atoms, and

further to provide such stationary phases that can be readily, efficiently and economically derivatized with a wide range of chemical functionalities.

A number of known limitations of the prior art using available chromatography stationary phases arise out of difficulties in the preparation of stationary phase materials having particular surface chemistries. Thus, for example, the range of organic polymers that can be derivatized for use as chromatography stationary phases, and the range of chemical functional groups that can be introduced onto the surfaces of such polymeric solid supports, may be restricted because chemical reaction conditions destroy or degrade the support material.

It is an aspect of the present invention to provide a mild method of introducing a chemical functionality onto the surface of an organic polymeric chromatography stationary phase such that essentially any organic polymer having hydrogen atoms may be surface functionalized according to the invention.

Certain organic polymers known in the art are not regarded as useful solid supports for chromatography because desirable functionalities cannot be introduced in sufficient concentrations. Another limitation in the art is the inability to control chemical reactions for functionalizing a solid support, whereby significant amounts of an expensive functional group (for example, a recombinant protein receptor fragment for affinity chromatography) that is desired on sample-accessible surfaces are wasted by becoming coupled to the solid support on sample-inaccessible surfaces. It is also an aspect of the present invention to provide improved efficiency and controllability in the surface functionalization of chromatography solid supports.

Accordingly, the present invention provides compositions and methods for separations of soluble molecules in liquid chromatography and more specifically, readily and controllably functionalizable solid chromatography supports formed from organic polymer. The present invention also provides a method for introducing functionality onto the pore surfaces of any porous chromatography stationary phase, and further provides porous solid chromatography supports having surface functionality distributed throughout the pore surfaces of the supports. The surface-functionalized porous stationary phases may be prepared using remote plasma discharge, as described herein.

With regard to chromatographic applications, the following terms have the indicated meanings:

"Solid stationary phase for liquid chromatography" means essentially any solid synthetic, semisynthetic or naturally occurring organic polymer having hydrogen atoms.

"Ion exchanger" means any charged chemical functionality that can be immobilized on an insoluble stationary phase matrix and that can reversibly associate with mobile counter-ions without altering the stationary phase matrix.

5 "Anion exchanger" means any ion exchanger that can reversibly associate with mobile counter-ions that are anions. Typical anion exchanger functionalities are aminoethyl, diethylaminoethyl, and quaternary aminoethyl.

"Cation exchanger" means any ion exchanger that can reversibly associate with mobile counter-ions that are cations. Typical cation exchanger functionalities are carboxymethyl, phosphor, and sulfopropyl.

10 "Hydrophobic group" means a group which is not water soluble. Typical hydrophobic functionalities are straight-chain alkyl of any length from methyl to C24, phenyl, etc.

"Hydrazide group" means -NH-NH-. Hydrazide groups may be used to couple glycoproteins to columns via their glycosyl moieties, which is useful where the only available amino groups are too close to the part of the protein that must interact with sample, *e.g.*, some monoclonal antibodies .

15 "Reactive group for covalent bond formation through protein amino groups" means any activated or activatable group that can form a covalent bond through available nitrogen atoms in amino groups on protein. Such groups are well-known to those familiar in the art and include CNBr activation for coupling to -OH groups, epoxy groups, dimethylpimelimidate or others (*see* Means and Feeney, "Protein Modification" and especially see Pierce catalog pp. 351-368 for a host of coupling strategies for proteins.

20 "A substituent group for reversed phase chromatography" means any hydrophobic chemical group that can be used as a selective adsorbent functionality for a sample in a mobile phase, where the mobile phase comprises an aqueous mobile phase containing a water-miscible organic solvent, such that altering the composition of the mobile phase alters its selectivity for the sample.

25 "A substituent group for chiral chromatography" means any chiral molecule that exhibits different affinities for two molecules in a sample that are enantiomers of one another. Examples are found in the catalog distributed by Regis Company.

30 "Multivalent" refers to a chemical moiety that has multiple reaction sites. Examples include tentacle, fimbriate, brush, starburst, dendrimer, pellicular, branched, etc. structures.

The particular needs of the chromatographic separation will determine the required configuration of the stationary phase in a liquid chromatography apparatus, and hence the required configuration of the porous article for a given chromatography application. Solid supports for chromatography according to the present invention may
5 be provided as porous or non-porous particulate material packed into cylindrical columns, as macroporous molded polymeric plugs, as planar, stacked or coiled membrane sheets placed in suitable housings, as fibrous matrices including tortuous path filters and hollow-fiber filters, or as any number of other solid supports for chromatography.

10 Among the critical parameters that may influence the suitability of a particular stationary phase configuration for a particular chromatographic separation include the sample capacity of the stationary phase, the resolution (degree of purification) that is required, the speed with which a separation must be accomplished, the physicochemical properties of the sample and various additional factors. For
15 example, solid supports that are particulate in nature often require careful packing into cylindrical columns for chromatography, or may be provided embedded within fibrous membranes, where in either case resolution can suffer because spaces between the particles contribute to unwanted bandspreading. The present invention provides a solid support for chromatography that may have essentially any shape, including but not
20 limited to the shape of a cube, block, sphere, tube, rod or cylinder, sheet, disc, membrane, film or the like.

Accordingly, chromatographic stationary phases of the invention may also, but need not, be particulate in nature. Non-particulate solid supports, *i.e.*, monolithic solid supports, can have desirable chromatographic properties that will be
25 appreciated by those familiar with the art. As discussed above, articles in the form of a block/cube, rod/cylinder, or a film are preferred for many applications. Moreover, the article may be a composite of two polymers, as described above.

A. SURFACE-FUNCTIONALIZED POROUS SOLID SUPPORTS FOR CHROMATOGRAPHY

The chromatographic supports of the present invention are preferably
30 prepared by contacting a precursor solid support with reactive gas-phase radicals, as discussed above. The surface chemistry of the solid support is very influential in determining the success of a chromatographic separation. This surface chemistry provides the primary interaction with sample that is the basis for separation of solutes in the sample.

For example, chromatographic separations on the basis of the molecular size of sample components typically involve little or no adsorption of sample to the stationary phase and so require chemically inert solid supports. Such size-based separations rely upon diffusion of sample molecules into and out of pores of varying sizes in the stationary phases. Thus sample retention is a function of the pore sizes of solvent-accessible spaces in the stationary phase relative to the Stokes radius of each sample component. Accordingly, large sample components that are too big to enter any pores remain in the mobile phase flow path and are swept through the interstices in the chromatography solid support by convective forces. This process thereby separates large solutes from intermediate sized solutes that may diffuse into some but not all pores before diffusing back into the mobile phase as it flows through the support and is collected as effluent. These components are further separated from small sample components that may diffuse into even the smallest solvent-accessible pores in the stationary phase, thereby being retained for longer times before diffusing back out to be collected in the effluent.

The present invention provides solid supports for chromatography that have suitable surface chemistry for such non-adsorptive chromatographic separations. In particular, the invention provides solid supports for liquid chromatographic separation of biomolecules, where these solid supports have surface chemistry exposed to the mobile phase that eliminates non-specific adsorptive interactions between the stationary phase and the sample components whose chromatographic resolution is sought. Such surface chemistries are achieved through introduction of amino or hydroxyl groups to the solid surface by remote plasma discharge as described above, optionally followed by subsequent reaction of the introduced amino and hydroxyl groups with various chemical reagents that amplify those functional groups and/or convert those functional groups into chromatographically active chemical groups such as cations, anions, and other groups that will selectively interact with solute, as discussed in more detail below.

The present invention provides solid supports having suitable surface chemistries for a variety of adsorptive chromatographic separations. Those familiar with the chromatography arts know that significant separation of components in a sample can be achieved by adsorptive chromatography, in which a sample molecule in a mobile phase gains access to stationary phase surfaces through diffusive and convective events as described above, and whereby such a sample further reversibly and non-covalently binds to a stationary phase by virtue of particular interactive forces. These forces derive from the physicochemical properties of the sample, the stationary phase,

and the mobile phase, including but not limited to hydrogen bonding, van der Waals forces, steric forces, electrostatic attractions, pH effects, hydrophilic and hydrophobic forces and other forces that may cause a solute to associate with a stationary phase specifically or non-specifically.

5 Examples of adsorptive chromatography include but need not be limited to well known techniques such as ion chromatography, ion-exchange chromatography (anion exchange and cation exchange), hydrophobic interaction chromatography, partition chromatography, reversed-phase chromatography, chromatofocusing, chiral chromatography and affinity chromatography. The present invention provides
10 chromatography supports having surface functional groups that are suitable for each of these adsorptive chromatography applications.

 Persons skilled in the art appreciate that adsorptive chromatography techniques are especially powerful tools for the isolation, concentration and purification of distinct molecular components from a complex sample mixture. For instance,
15 adsorptive chromatography methods are particularly useful for purification of biomolecules.

 According to the adsorptive chromatography art, it is known to provide chromatography stationary phases having particularly desirable surface chemistries for interactions with sample in the mobile phase. For example, stationary phases for ion-
20 exchange chromatography may have strong or weak anion-exchanging or cation-exchanging functionalities available on mobile-phase accessible surfaces for interaction with sample. As another example, reversed-phase chromatography stationary phases may have alkyl chains of a particularly defined length available on mobile-phase accessible surfaces for interaction with sample. As another example, an affinity
25 chromatography stationary phase may have a particular high-affinity receptor (such as a monoclonal antibody that is specific for a sample component) available on mobile-phase accessible surfaces for interaction with sample. Many other desirable stationary phase surface chemistries will be known to the skilled artisan based upon the particular separation to be achieved using liquid chromatography. It is known in the art how to
30 effect the adsorption of a molecular species of interest to such a stationary phase under mobile phase conditions that cause the bulk of contaminants in the sample solution to not be retained by the stationary phase, and further how to subsequently modify mobile phase conditions in such a manner as to permit selective desorption of the sample species of interest from the stationary phase, and its elution in the mobile phase. Thus
35 according to the examples provided above, sample desorption in ion-exchange chromatography is achieved by altering the mobile phase composition to include

effective concentrations of a suitable counter-ion that may displace the sample, which is reversibly adsorbed to the solid support's surface functionalities by ionic forces, from the stationary phase. In the case of reversed-phase chromatography, sample desorption is accomplished by introduction into the mobile phase of organic modifiers or other agents that may alter the solvent strength or polarity of the mobile phase in such a manner as to discourage sample association with surface functionalities of the stationary phase and favor sample diffusion into the mobile phase. And in the case of affinity chromatography, it is well known that affinity interactions between a sample component and the stationary phase surface functionality may be reversed by altering the mobile phase composition by means of one or more of an effective pH change, an added chaotropic agent, high salt concentration, and a suitable concentration of a specific, high-affinity competitive inhibitor that displaces the sample from binding sites on the stationary phase surface.

The porous article having surface functionality according to the present invention may be obtained by treating a porous article as described above with remotely-generated gas-phase radicals, also as described above. The porous article has an external surface, a bulk matrix, and pores which extend from the external surface into the bulk matrix. The pores are surrounded by, and thus define, the pore surface. The bulk matrix and surface of the (pre-treated) article is formed, at least in part and preferably in whole, of organic polymer, *i.e.*, polymers having carbon and hydrogen atoms.

As discussed above, surface-functionalized porous articles can be characterized using standard methods. The functional groups that are added to a surface according to the invention are hydrophilic. Thus, after sufficient functionalization of a hydrophobic polymer surface, all points on the surface may be made hydrophilic. However, the functionalization will not extend into the bulk matrix, but instead will be confined to the surface or, at most, the near surface region, so that the matrix is free from the hydroxyl, amino, etc. functional groups that have been added to the surface.

In a preferred embodiment of the chromatographic application of the present invention, a hydrophobic porous polymer article is treated with a substantially uniform concentration of reactive radicals, either atomic oxygen, hydroxyl or amino radicals, using remote plasma discharge to form a substantially uniform hydrophilic surface layer on the article. In a more preferred embodiment, the hydrophilic surface substantially comprises reactive hydroxyl functionality, while in another more preferred embodiment, the hydrophilic surface substantially comprises reactive amino functionality. The surface functionality is spread across the entire surface of the article,

which includes the external surface and the pore surface. Complete surface functionalization can be detected and distinguished from partial surface functionalization by wicking experiments with water, at least when the untreated surface is hydrophobic.

5 B. CHROMATOGRAPHY SUPPORTS HAVING SPACER GROUPS

According to the methodology described above, the invention provides modified porous article wherein non-native surface-bonded oxygen and/or nitrogen atoms are also bonded to one or more functional groups to form new molecular and supramolecular structures on the surface of the precursor article. In Structures I and II,
10 the functional groups "f" are chromatographically active, in that they will interact with solutes and/or materials dispersed in a liquid phase, so as to permit separation of different solutes and materials. The linker groups L will typically afford a large number of chromatographically active "f" sites, even though L is only bonded to one or a few X sites on the solid support. Exemplary amplification chemistry is illustrated in Figures
15 3A and 3B.

Amplification chemistry can be used to provide any of a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase and a fractal polymer phase to the surface of the chromatography support. Amplification chemistry can provide a grafted polymer having a plurality of amine or
20 hydroxyl groups attached to the solid support. These various phases will incorporate chromatographically active functionality, to thereby enhance the ability of the chromatography support to separate solutes and the like. Examples 4 and 5 herein disclose preferred examples of amplification chemistry. As will be seen in these Examples, amplification may be achieved by covalent linkage of a polyfunctional
25 material to the surface of the solid support.

C. DEVICES FOR CHROMATOGRAPHY

Another aspect of the invention is devices designed to capitalize on the advantages of the chromatography supports of the invention. One such device is illustrated in Figure 4, which is described above in relation to solid phase synthesis
30 applications. For chromatographic applications, Figure 4 shows a cassette comprising a cylindrically-shaped monolithic chromatography support of the invention, inserted into the barrel of a housing, where the housing has a length (l) and a diameter (d). The chromatography support may be a stack of discs, rather than being monolithic.

However, because the chromatography support fits snugly within the housing, and is either monolithic or formed of a plurality of stacked discs, there is no need for frits at either end of the reaction chamber: no particulates are either present within the chromatography support or will break free therefrom. In order to provide a connection to tubes at either end of a reaction chamber, each end is tapered to a Luer configuration. This is especially convenient when the device will be fitted into an automated chromatography device, *e.g.*, an HPLC column, for example, Perkin-Elmer or the like.

The device of Figure 5 is well-suited for larger scale chromatographic separations, in which a chromatography support of the invention fitted within a housing. The housing has two end pieces, through which solutions enter and exit, respectively. The housing also has side surfaces, to help retain solutions within the solid support. The end housing through which solutions enter preferably has a labyrinthine design to permit better distribution of flow across the separation chamber. The end and side housings may be made of plastic, *e.g.*, polyethylene, which can snap together to form a liquid-tight seal.

The monolithic nature of a preferred chromatography support of the invention provides many advantages which are recognized in the parts of the device shown in Figure 5. For instance, there are no end frits, plungers or the like to retain the particulate material that is commonly employed in reaction and separation columns. Furthermore, the device makes no accommodation for the user to gravity pack a column, because the chromatography support is simply inserted into the housing. While the chromatography support is illustrated as a single piece, in fact it may be formed of more than one short piece, where the shorter pieces are stacked one on top of another to fill the housing.

As described above, the functionalized polymeric articles of the invention are useful as solid supports for liquid chromatography. However, the articles may be used in a number of other applications including, but not limited to, solid-phase organic synthesis, including the synthesis of biomolecules, filtration media, carriers for immobilizing bioreactive components in bioreactor applications, gas chromatography media, media for distillation or extraction processes, and supports for diagnostic assays.

4. Use of Porous Articles in Solid Phase Assays

Assays for diagnosing and/or monitoring diseases commonly involve the detection of one or more molecular markers within a sample obtained from a patient. To perform such assays, a diagnostic agent that binds to a target molecule of interest is

often immobilized on a solid support, such as a bead, membrane or microtiter dish. The immobilized agent is then contacted with the sample and allowed to bind to the target molecule, which facilitates separation of the target molecule from the remainder of the sample. Following separation, the target molecule is generally detected using a reporter group and well known techniques.

Ideally, a solid support for diagnostic applications will have a high surface area with hydrophilic and/or chemically reactive character, good physical properties (*e.g.*, strength and moldability) and a low manufacturing cost. The solid supports currently employed within diagnostic applications are generally deficient in one or more of these criteria. As a result, assays often require long sample equilibration times and specialized equipment, such as low speed orbital motion tables and plate washers, which hampers adaptation to home testing products.

The present invention provides methods, assay devices and kits for detecting a variety of target molecules. In particular, the methods described herein are useful for diagnosing and monitoring patient conditions and diseases. Such methods employ a diagnostic agent that is immobilized on a surface-functionalized polymeric article prior to contact with a sample to be assayed. For *in vitro* assays, following contact with the sample and binding of the target molecule to the immobilized diagnostic agent, the target molecule may be separated from the remainder of the sample and detected using any of a variety of standard techniques. As discussed in greater detail below, the supports (or "articles") employed within the present methods possess good physical properties and a high surface area with reactive and/or hydrophilic functional groups distributed throughout. Diagnostic assays that employ such articles generally display enhanced efficiency and selectivity due to the unique properties of the surface-functionalized articles.

Surface-functionalized polymeric articles for use in solid phase assays of the present invention generally have the following characteristics:

(1) The articles comprise an organic polymer bulk matrix (*i.e.*, the volume of article that does not include the surface), an exterior surface (*i.e.*, the surface that is visible using a microscopic technique such as scanning electron microscopy without cutting or sectioning the article) and pores having interstitial surfaces (*i.e.*, surfaces that surround and define the pores).

(2) At least one hydrophilic and/or chemically reactive surface functionality is distributed, as a result of surface modification, throughout the exterior and interstitial surfaces. The surface modification includes, and may consist entirely of, replacing some fraction of the hydrogen atoms from some of the organic polymer's C-H

bonds with either oxygen or nitrogen atoms, so as to form C-OH, C=O, COOH and/or C-NH₂ functionalities.

(3) The functionalized article does not display appreciable surface roughness, according to SEM analysis, relative to the unmodified article.

5 (4) The pore volume of the functionalized article is not significantly altered (*i.e.*, surface-functionalized porous articles may have pore volumes that are within 10%, and preferably within 5%, of the pore volume of the unmodified article).

The surface functionalities are introduced by covalent attachment into the basic structure of the organic polymer(s) which form the surface of a solid article. In
10 this way, the surface of a polymeric article (or selected portions thereof) becomes hydrophilic and/or reactive due to a permanent change in the chemical composition of the surface region, rather than merely by virtue of being covered or otherwise masked with hydrophilic moieties. This functionalization does not extend into the bulk matrix, but instead is confined to the surface (and, in some instances, the near surface region).
15 In other words, organic polymer much below the surface of the functionalized article has the same carbon framework as the surface polymer, absent the oxygen or nitrogen atoms introduced by treatment. Preferably, the surface functionalization does not introduce functional groups below about 1000 angstroms from any polymer surface. More preferably, there are no non-native (*i.e.*, no non-constitutive) oxygen or nitrogen
20 atoms below about 100 angstroms, and still more preferably below about 10 angstroms from the surface. The oxygen or nitrogen atoms typically provide at least about 0.01 micromoles of reactive functional group per gram as measured by molecular probe methods, or 1 atom percent R-OH or N-NH₂ as measured by XPS methods.

It is possible to limit functionalization to a portion of the article surface,
25 by masking or by limiting the duration of exposure to the gas phase radicals. Generally, the diffusion of the gas-phase radicals from the exterior surface through the interstitial volume of the article proceeds in a distinct front. Behind the front, the interstitial surfaces of the pores become functionalized by the introduction of amino, hydroxyl and/or other groups. Ahead of the front, the pore surface retains its initial structure and
30 functionality. When the reaction front is allowed to pass through the entirety of the article, the entire surface of the article gains functional groups. Both fully and partially functionalized articles may be used within diagnostic methods as described herein, but the use of fully functionalized articles is generally preferred for most applications.

Preferably, the polymer is inherently hydrophobic. In general,
35 hydrophobic polymers which may be used according to the present invention are well known in the art, and are listed, for example, in Brandrup, J. et al. (eds), *The Polymer*

Handbook, 3rd ed., John Wiley & Sons, Inc. (New York, 1989). Hydrophobic polymers include hydrocarbons such as polyethylene (low density, high density and ultra high molecular weight), polypropylene, polybutadiene, polystyrene and poly(co-styrene divinyl benzene); PTFE; polyacrylonitrile; polyetherimide; polysulfone and polyethersulfone. Polyethylene is a preferred hydrocarbon, with ultra-high molecular weight polyethylene (as described, for example, in U.S. Patent No. 5,531,899) particularly preferred.

Preferred hydrocarbon polymers are polyolefins. Suitable hydrocarbon olefins from which a porous article can be prepared include, but are not limited to, ethylene, propylene, butylene, butadiene, styrene, α -methylstyrene, divinylbenzene and the like, although preferred hydrocarbon olefins do not contain aromatic rings.

As discussed above, the polymer may contain heteroatoms such as a halogen, silicon, sulfur, oxygen and/or nitrogen atoms. Moreover, the article may be a composite of two or more polymers. Similar to other applications described herein, a porous article of an assay may have essentially any shape. For example, an article may be in the shape of a cube, block, sphere, tube, rod or cylinder, sheet, disc, membrane, film, monolith or the like. Such a shape may result from drawing, molding, sintering and/or one or more other polymer processing steps.

In a preferred embodiment, a hydrophobic porous polymer article is treated with a substantially uniform concentration of reactive radicals (atomic oxygen, hydroxyl or amino radicals) using remote plasma discharge to form a substantially uniform hydrophilic surface layer on the article. In a more preferred embodiment, the hydrophilic surface substantially comprises reactive hydroxyl functionality, while in another more preferred embodiment, the hydrophilic surface substantially comprises reactive amino functionality. The surface functionality is spread across the entire surface of the article, which includes the exterior surface and the pore surface. Complete surface functionalization can be detected and distinguished from partial surface functionalization by wicking experiments with water, at least when the untreated surface is hydrophobic.

A. IMMOBILIZATION OF DIAGNOSTIC AGENT

For diagnostic applications, a diagnostic agent is typically immobilized on the surface of a functionalized polymeric article. As used herein, a "diagnostic agent," is any compound, cell or organism that binds to the target molecule of interest. A target molecule may be any compound (or marker) whose level within a body fluid or tissue aids in the diagnosis of a patient's condition or in monitoring disease progression

or therapy. Alternatively, a target molecule may be a molecule that is generated *in vitro* within a reaction designed to determine the level of activity of a compound of interest (e.g., the activity of a kinase within a sample may be determined by detecting the level of phosphorylated substrate following an *in vitro* kinase reaction). Many target
5 molecules are currently known, and many more are being discovered. Common target molecules include antigens (e.g., prostate-specific antigen), antibodies (such as antibodies raised against pathogenic proteins), polynucleotides (e.g., mRNAs encoding disease-associated proteins, such as tumor-associated proteins), substrates, receptors and ligands.

10 Diagnostic agents may be naturally occurring, recombinant, synthetic or any combination thereof. Preferably, the agent selectively binds the target molecule (i.e., has an affinity for the target molecule that is sufficient to allow detection within one or more of the representative assay formats described herein, without binding to other components of the sample at a level that interferes significantly with the
15 functioning of the assay). More preferably, the diagnostic agent has an affinity for the target molecule of at least about 10^2 L/mol. Affinity constants may generally be determined using standard techniques.

While a diagnostic agent may be any of a variety of substances, certain compounds generally have the greatest utility. For antigen target molecules, a
20 diagnostic agent is commonly an antibody. Antigens often are suitable diagnostic agents for detection of antibody target molecules. A polynucleotide target molecule may be detected using a complementary polynucleotide probe. Receptors and ligands, or enzymes and substrates, also may be suitable as target molecule/diagnostic agent pairs.

25 One or more diagnostic agents may be immobilized on a functionalized polymeric porous article using any of a variety of well known techniques. Within the context of the present invention, the term "immobilization" refers to association via noncovalent interactions, such as adsorption, as well as covalent attachment. Adsorption may generally be achieved by contacting the diagnostic agent with the
30 functionalized article, in a suitable buffer, for an appropriate amount of time (which may vary with temperature, but is typically between about an hour and a day). Suitable conditions that favor adsorption are well known in the art.

Covalent attachment of a diagnostic agent may be a direct linkage to a surface functional group (e.g., via a condensation reaction), or may be achieved via a
35 linker (or cross-linking agent) or an amplifying group. Linkers are typically bifunctional reagents that react with both the support and a functional group, such as a

hydroxyl or amino group, on the diagnostic agent. Suitable linkers are generally well known. An amplifying group may be interposed between the surface oxygen and/or nitrogen atom and the diagnostic agent, such that a plurality of diagnostic agents are bonded to the amplifying group.

5 A preferred linker is trichloro-s-triazine, which in acetone or other suitable solvent and in the presence of aqueous bases such as dilute aqueous NaOH or a 0.05 molar sodium carbonate buffer, pH 9.0, will, at low temperature (4°C) react with an aminated or hydroxylated article to afford a linker moiety having two chloride groups. These chloride groups may be reacted with polyethylene glycols of low
10 molecular weight (such as are available from Shearwater Polymers of Huntsville, AL) having hydroxyl or amino groups at one end and sulfonated or quaternized amines on the other end, to provide a tenticular structure. Alternatively, these chloride groups may be reacted with polyfunctional amines (*e.g.*, TREN from Pressure Chemical, Pittsburgh, PA and poly(allylamine) from Aldrich Chemical, Milwaukee, WI).

15 Coupling agents such as toluene sulfonyl chloride may also be used to link amine-containing diagnostic agents to the surface-functionalized article by first reacting the toluene sulfonyl chloride with the R-OH groups of the article (in an aprotic solvent such as acetone with an aprotic base catalyst such as pyridine) and then reacting the resulting surface sulfonic esters with amines to produce alkyl amine surface
20 linkages.

To amplify the hydroxyl and/or amino groups, such groups may be reacted with multi-functional reagents, as described above. Figures 3A and 3B, for example, illustrate the grafting of polymers on to a solid support.

In some cases, the linkers or amplifying groups have the same structure
25 as the surface functional groups. This may be the case, for example, when polyfunctionalized polymers are grafted onto a hydroxylated or aminated solid support. For example, dendrimers having a plurality of functional groups may be used. Preferred dendrimers are Starburst™ dendrimers, hyperbranched dendrimers and fractal-polymer phases. Dendrimers provide stationary phases with high degrees of
30 surface functionalization while being stable against surface restructuring with polar group involution. The dendrimers not only provide a high degree of functionality, but they also allow for a controlled microporosity to be placed over the surface of the solid support. Dendrimers may be combined with other linker or amplifying groups to impart a very high degree of functionality to the surface of a solid support.

35 Following immobilization of the diagnostic agent, any remaining binding sites may be blocked with any suitable blocking agent known to those in the art

(e.g., bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO)). The immobilized agent may then be used within a diagnostic assay, as described below.

The immobilization of specific antigens, antibodies, polynucleotides, enzymes or other selective binding or reacting agents including supramolecular self-assembly reagents has been discussed in numerous references, including U.S. Patent Nos. 5,141,806, 5,332,551, 5,369,012, and 5,314,857.

B. DIAGNOSTIC ASSAYS

As noted above, the surface-functionalized porous articles may be used as supports for immobilized diagnostic agents in assays to detect a particular target molecule within a sample. For diagnostic purposes, the sample is generally obtained from a patient. Suitable samples may be biological fluids or tissue samples, and will depend on the target molecule and diagnosis of interest. Representative samples include, but are not limited to, blood, serum, urine, saliva, sputum, bronchial aspirates, breast duct aspirates, feces, cervical secretions, synovial and cerebrospinal fluid, intestinal irrigate, gastric fluid, and tissue samples. A sample may, but need not, be diluted with a suitable buffer (e.g., PBS) prior to incubation with the immobilized diagnostic agent.

A target molecule may be any of a variety of sample components such as, for example, an antigen, antibody, polynucleotide, ligand, enzyme or receptor. A target molecule may be associated with the surface of a cell or a pathogen, thereby permitting the detection of a cellular or pathogenic target. In certain specific embodiments, the target molecule may be a ubiquitin protein or prostate specific antigen.

For detection and/or quantitation of a specific target molecule, a sample is combined with a functionalized polymeric article coated with a diagnostic agent having affinity for the target molecule. The target molecule is allowed to react with the diagnostic agent for an incubation time sufficient to permit the detection of target molecule. Preferably, the incubation time is sufficient to achieve a level of binding that is at least 90%, more preferably 95%, of that achieved at equilibrium. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium (or a given fraction thereof) may be readily determined by assaying the level of binding that occurs over a period of time. Following incubation, the target molecule is generally separated from any non-bound components (and washed with an appropriate buffer, as needed) and detected by conventional methods. The use of a functionalized porous polymeric article, as described herein, increases the effective concentration of

diagnostic agent and decreases equilibration and washing times, resulting in improved assay efficiency and sensitivity.

Any assay format known in the art may be employed including, but not limited to, immunoassays, receptor binding assays, enzyme assays and hybridization assays. Immunoassays are useful for detecting very small amounts of target molecule, and may be performed using a radioimmunoassay (RIA), enzyme mediated immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) method (*see e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). Such assays may be performed in a sandwich assay format or in a competitive binding format. Within one embodiment, the immobilized diagnostic agent may be an antigen capable of binding to, and detecting the presence of, antibody in a sample. Following separation from the remainder of the sample (and washing as needed), bound antibody may be detected using a detection reagent that binds to the antibody/antigen complex and contains a reporter group. Alternatively, an antibody may be immobilized and used to detect the presence of antigen in the sample. Receptor/ligand, enzyme/substrate and complementary nucleotide pairs may also be used as diagnostic agent and target molecule in a similar manner.

Suitable detection reagents may comprise a binding component (*e.g.*, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group (*e.g.*, an enzyme, substrate, cofactor, inhibitor, dye, radionuclide, luminescent group, fluorescent group or biotin). The conjugation of a binding component and a reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common detection reagents may also be purchased from many commercial sources (*e.g.*, Zymed Laboratories, San Francisco, CA and Pierce, Rockford, IL).

Following removal of unbound detection reagent, bound detection reagent may be detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme. Enzyme reporter groups (*e.g.*, horse radish peroxidase) may generally be detected by the addition of substrate (generally for a specified period of time), followed by spectroscopic or other analysis of the reaction products.

Alternatively, a competitive assay may be utilized, in which an antibody that binds to the target antigen is labeled with a reporter group, is incubated with the sample, and is allowed to bind to the immobilized antigen. The extent to which components of the sample inhibit the binding of labeled antibody to the immobilized antigen may be detected as described above, and is indicative of the level of target antigen within the sample.

Immunoassays may also be performed using a flow-through or strip test format. In this embodiment, one or more antigen or antibody diagnostic agents may be immobilized on a functionalized porous article in the form of a membrane. In a flow-through test, target molecule within a sample binds to the immobilized diagnostic agent as the sample passes through the membrane. A detection reagent as described above then binds to the target molecule/diagnostic agent complex as the solution containing the detection reagent flows through the membrane. The level of bound detection reagent may then be determined as described above. In the strip test format, one end of the membrane is immersed in the sample. The sample then migrates along the membrane through a region containing detection reagent and to the area of immobilized diagnostic agent. Concentration of detection reagent at the site of the diagnostic agent (typically in a visually discernible pattern) indicates the presence of target molecule in the sample, and the absence of such concentration indicates a negative result.

Diagnostic assays may be qualitative, semi-quantitative or quantitative. The use of standards and standard additions for semi-quantitative and quantitative assays is well known in the art. For qualitative assays to determine the presence or absence of a particular condition, the level of signal detected may be compared to that of a predetermined reference value having diagnostic significance. For example, when the target molecule is a marker for a given disease, the reference value may be the level of target molecule found in samples from individuals who are not afflicted with the disease. In such instances, the extent of change in the level of target molecule that is sufficient to indicate the presence of the disease will depend upon the specific assay. In general, however, a sample generating a signal that is at least one standard deviation higher or lower than the reference value may be considered to have an altered level of target molecule, indicative of the disease. Preferably, the level of target molecule changes by at least three standard deviations as a result of the condition being diagnosed.

The functionalized porous articles described herein are also useful as visual probes or markers of pathology in a histological sample. For this purpose, the immobilized diagnostic agent is specific for a target molecule expressed during a

particular pathologic condition. For example, the diagnostic agent may be an immunoglobulin, a cell receptor or an oligonucleotide probe specific for an abnormal cell, such as a rapidly proliferating cell, or a pathological organism such as a virus. The diagnostic agent may be labeled with a detectable label, or may be detected using an indicator reagent comprising a detectable label. The label is then detected using a suitable method, which will depend upon the nature of the label and will be apparent to those of ordinary skill in the art.

The functionalized porous articles described herein may also be used as imaging agents for *in vivo* localization of a particular molecule, cell type or pathologic condition in a manner similar to that described above for histopathology. Within this embodiment, a pathological condition may be detected, or the effect of a therapy may be monitored. For this use, the functionalized porous articles are generally administered to the patient in the form of a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous suspension or emulsion, which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Representative carriers include physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Optionally, a pharmaceutical composition may additionally contain preservatives and/or other additives such as, for example, antimicrobial agents, antioxidants, chelating agents and/or inert gases, and/or other active ingredients. After administration, and passage of sufficient time to allow localization to the affected organ or region of the body, the immobilized diagnostic agent binds to the target molecule and can be detected using conventional imaging techniques, such as x-ray technologies.

Numerous other assay protocols exist that are suitable for use with the functionalized porous articles described herein. The above descriptions are intended to be exemplary only.

In a related aspect of the present invention, kits for detecting one or more target molecules as described herein are provided. In general, such kits comprise a diagnostic agent immobilized on a surface-functionalized porous article, as described herein within one container. One or more additional containers enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also contain a detection reagent that contains a reporter group suitable for direct or indirect detection of the bound target molecule.

C. POROUS SOLID MICROREACTORS

Within one embodiment, a diagnostic assay may be performed using a device comprising a two or three dimensional array of porous solid microreactors (μ -Rs) capable of receiving sample and reagent liquids and taking up those liquids by capillary action combined with gravity-driven flow. Each μ -R is typically in the form of a sheet or plug, and comprises one or more porous polymeric articles optimized for specific assay applications so as to minimize nonspecific absorption or other nonspecific reactions. Within such a device, each μ -R is separated from the others in two dimensions, x and y, such that cross talk between μ -Rs is eliminated. If desired, the microreactors may be in register and in contact with each other on the third dimensional axis, z, so that flow from one microreactor to the next results when one two dimensional microreactor array is brought into contact with another.

Sample, wash and reagent solutions can be added to a μ -R such that fluid uptake and retention are governed by capillary action and gravity. While each μ -R element is wetted by the sample, reagent and wash fluids, the structure holding the μ -R array in register is not intended to prevent sample cross talk.

An additional component of the device may be an absorbent towel or sheet used to draw fluid out of the μ -R array as needed, again by a combination of capillary action and gravity flow. The results of a particular assay conducted with the μ -R system may then be read out using one of a variety of methods familiar to practitioners of the art, such as optical multi-well-plate readers, fluorescence imaging or visual inspection. The read out may follow washing of the μ -R contents into a conventional multi-well-plate or spectrometer cuvette or scintillation vial or other well known instrument liquid sample holder, or the μ -R may be read directly.

The use of such a device permits the detection and/or measurement of target molecules with enhanced efficiency, sensitivity and selectivity. Such a device presents an optimal surface area for reagent immobilization and permits the use of reduced sample and reagent volumes. Equilibration of macromolecular assay systems is rapid, as a result of reduced diffusion lengths. In addition, fluid handling is simplified due to the use of capillary action in porous solid μ -R for sample and reagent uptake followed by simple blotting with superabsorbent polymer material or other absorbent material to remove fluid from μ -R (also by gravity flow/capillary action). Multi-step protocols can be implemented easily by stacking μ -R arrays with gravity/capillary action carrying sample and reagent fluids through different μ -RS in series or in parallel.

The present μ -R array technology can be used, for example, whenever quantitative or semiquantitative concentration data is needed. In particular, this technology is applicable to measurements requiring titration curves, serial dilution assays and quantitative multifactor diagnostic testing. The microreactor has particular utility as an immobilized enzyme reactor array for clinical assays, and as an affinity ligand reactor for ELISA and related measurements.

By way of example, a representative μ -R array may be formed consisting of an array of hydrophilic volume elements in an otherwise hydrophobic solid sheet. Such an array may be formed from a porous solid sheet having a contiguous open pore structure that is capable of reacting with gaseous atomic oxygen to produce a hydrophilic surface. The array of hydrophilic volume elements may be prepared by masking the porous solid sheet such that reactive gasses reach the outer surface of the porous solid sheet and enter the pore volume by diffusion only where it is desired to create a μ -R. Both sides of the sheet may be masked with the mask openings aligned in register. Alternatively, a μ -R array may be prepared by inserting porous solid plugs into an inert, rigid hydrophobic sheet made of, for example, Lexan, polyethersulfone, polystyrene, polyethylene or polypropylene.

A μ -R array contains distinct regions of hydrophilic and reactive character where a diagnostic agent may be immobilized as described above. Preferably, each μ -R is a contiguous hydrophilic region connecting both sides of the solid sheet. A μ -R array may be manufactured according to printed circuit manufacture methods to produce a porous 3-D array for compartmental flow and mixing, compartmental reactions and other applications useful for diagnostic assays.

In another embodiment, two or more two-dimensional μ -R arrays are stacked so that the first μ -R accomplishes a sample preparation step for the second by, for example, removing Ig-G selectivity in a rapid assay for Ig-E. A device containing one or more arrays may be prepared using conventional techniques from any number of materials, as delineated above, and including LEXAN, polyethersulfone, polystyrene, polyethylene or polypropylene.

As an additional embodiment, three microreactors may be configured on a single test strip or stick for testing, such as at-home self-testing, such that one μ -R is for the negative standard, one for the positive standard and one for the user's sample.

As a further embodiment, gravity feed and capillary flow are only used in the sample application step, and the μ -R array is assembled or fabricated in a structure compatible with filter holds using syringe driven fluid flow such that all steps

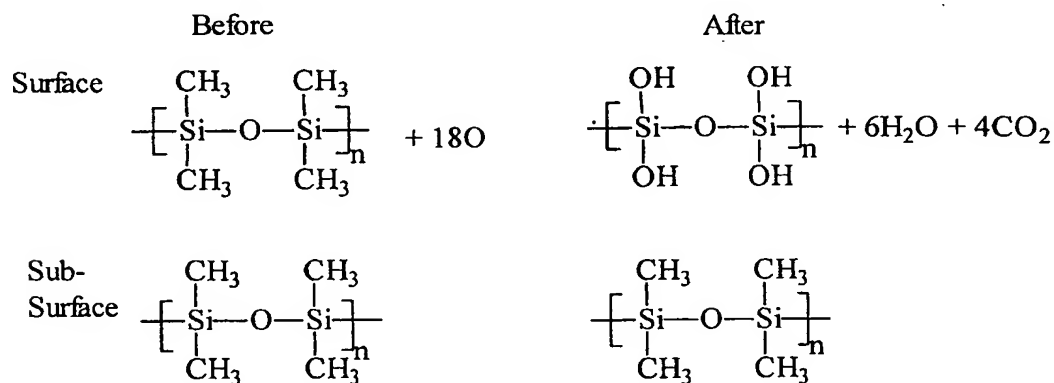
following sample application and blotting involve manual or powered syringe driven flow.

Regardless of the precise configuration of the device, sample or standard solution may be added to each μ -R, allowed to react briefly and then removed by, for example, capillary flow and absorption into an absorbent material. Washing buffers may be similarly added and removed by, for example, capillary and gravity driven flow or syringe driven flow, to remove non-specifically absorbed substances. Finally, an indicator reagent may added to produce, for example, a colorimetric, fluorometric or chemiluminescent reaction which can then be read using photometric instrumentation well known to practitioners of the art. The indicator reaction can be read directly in the porous solid μ -R array or washed through each μ -R into a conventional multi-well plate for readout. Chemical sensor technology and microelectronic sensors can also be used.

The following specific examples serve to further illustrate the invention. These examples are merely illustrative of the invention and are not to be construed as a limitation thereof.

EXAMPLE 1

A polytetrafluoroethylene reinforced silicone membrane sold under the trade name SILON by Bio-Med Sciences, Inc. of Bethlehem, PA is uniformly treated with atomic oxygen in an atomic oxygen reactor having a specimen holding sidearm. Operating conditions are shown in Table 1. The membrane initially has a hydrophobic surface which after treatment acquires hydrophilic hydroxyl functionality as follows:



Pressure (Pa)	27
Temperature (K)	298
Gas Mixture	10% O ₂ in Ar
C(z) (AO/cm ³)	4 x 10 ¹⁴

EXAMPLE 2

Discs of porous and nonporous high density polyethylene (HDPE) were treated with atomic oxygen to evaluate mass loss and the development of hydrophilicity. The porous polyethylene discs were cut from 1.6 mm (1/16-in.) thick specimens obtained from Porex Technologies (Fairburn, GA) and had coarse, medium and fine pores with respective nominal pore diameters of about 250 μm , 49-90 μm , and 10-20 μm . The nonporous HDPE was obtained in the form of a 0.025 mm (1 mil) film. The discs had a diameter of about 19 mm (0.75 in.) and were cleaned by rinsing in a mixture of 1,1,1-trichloroethylene and ethanol and drying in a vacuum overnight.

Duplicate sets of the discs were placed in the sidearm reactor of Example 1 and exposed to an atomic oxygen concentration of $5 \times 10^{14}/\text{cm}^3$. The weight of each disc was recorded at periodic intervals over a total exposure time of 12-15 hours to monitor mass loss. The results are presented in Table 2. In addition, scanning electron micrographs (SEM) of discs exposed to $3.6 \times 10^{14}/\text{cm}^3$ atomic oxygen atoms in the sidearm reactor for 166.25 hours were taken for comparison to unexposed material. As seen from the data in Table 2, the rate of mass loss varied according to material pore size, *i.e.*, the larger the pore diameter, the greater the mass loss rate. Apparently, the mass loss rate depends mostly on the amount of surface area available for reaction, and not on diffusion. Estimates of the effective surface area of the discs calculated from the loss rate relative to the nonporous HDPE were corroborated from surface area estimates by SEM. The SEM also showed rounding of particles in the coarse pores and visible pitting in the medium and fine pores, with changes occurring uniformly across the thickness of the discs.

Discs were similarly exposed to $5 \times 10^{14}/\text{cm}^3$ atomic oxygen atoms in the sidearm reactor for periods of time ranging from three seconds to ten minutes to characterize development of hydrophilicity as reflected in the amount of water uptake of the exposed samples. An amount of water sufficient to wet the hydrophilic portion was dropped onto the surface of each disc, and any excess was removed by pipet. The

increase in mass due to water uptake was recorded, and the results based on an average of three specimens are presented in Table 3.

TABLE 2

Time (hours)	Sample Weight (mg)			
	HDPE Sheet (1 mil)		POREX, Medium (49-90 μm)	
	Disc #1	Disc #2	Disc #1	Disc #2
0.0000	6.8223	6.7433	226.8562	229.1452
1.833	6.4403	6.3288	225.2864	227.5929
4.0667	6.1962	6.0763	224.4133	226.7565
5.8834	5.9684	5.8701	223.6999	226.0678
7.8001	5.7616	5.6614	223.0172	225.3709
9.7834	5.5604	5.4508	222.3613	224.6970
11.7834	5.3590	5.2480	221.6863	223.9940
14.2834	5.1219	5.0195	220.9277	223.2801

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TABLE 2 (cont.)

Time (hours)	Sample Weight (mg)			
	POREX, Fine (10-20 μm)		POREX, Coarse (250 μm)	
	Disc #1	Disc #2	Disc #1	Disc #2
0.0000	254.0969	253.5941	451.5491	454.2949
1.6167	253.0543	252.7013	450.7072	453.0781
3.6167	252.4069	252.0329	449.8757	451.7606
5.6167	251.7920	251.4103	449.2417	451.1571
7.1000	251.2774	250.9041	448.5435	450.6771
9.1000	250.5556	250.1976	447.8187	449.7215
11.1167	249.9119	249.5267	446.7887	449.0990
12.8500	249.3742	248.9901	445.5790	448.0119

As seen from these data, the amount of water uptake increased linearly until the saturation level was reached. The development of hydrophilicity clearly occurred prior to observation of any mass loss, and appeared to be dependent on the rate of diffusion of the atomic oxygen through the porous solid. As the pore size increased,

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- the diffusion rate also increased. Also, dyeing of the specimens using a water soluble dye showed that a distinct reaction front progressed through the porous material. The partially hydrophilicized samples had a distributed pore chemistry, the portion ahead of the reaction front remaining hydrophobic while the portion behind the reaction front was hydrophobic.

TABLE 3

POREX, Fine (10-20 μm)		POREX, Medium (49-90 μm)		POREX, Coarse (250 μm)	
Time Exposed (sec)	Water Absorbed (% of disc wt)	Time Exposed (sec)	Water Absorbed (% of disc wt)	Time Exposed (sec)	Water Absorbed (% of disc wt)
5	1.9	3	5.2	2	4.6
10	8.4	5	6.7	4	14.0
15	10.4	10	12.1	6	17.3
20	11.6	10	12.7	10	25.8
30	14.0	15	18.9	15	36.5
40	18.0	20	18.9	15	36.5
50	20.1	30	34.0	22	47.3
70	23.2	40	41.8	40	53.4
90	24.3	60	48.0	60	54.1
120	32.9	90	64.8	90	52.6
180	47.0	120	68.0		
300	55.7	150	74.3		
600	65.9	180	75.0		
700	65.2	210	79.5		
		240	82.8		

EXAMPLE 3

- In a third example, 4 samples of a porous solid consisting of UHMWPE and having a 7 micron nominal pore size, and of 0.025 inches thickness, was exposed to ammonia FDRC conditions in a side-arm reactor of the type described above for periods of time ranging from 30 minutes to 3 hours and at temperatures of 1) 25 °C, 2) 50 °C, and 3) 80 °C, at a total pressure of 2.06 Torr with the plasma source on and 1.93 Torr with the plasma source off. At the end of the FDRC treatment period, the plasma

source is turned off and the porous material is further treated by a 30 minute soak in the ammonia - argon gas at a total pressure of 400 Torr. The reactor was essentially identical to the side-arm reactor described above. The mass flow rate of the 10 percent ammonia in argon working gas was 132 standard cubic centimeters of gas per minute.

5 The plasma source was an air-cooled Evenson cell operated at 70 Watts of forward RF power and 3 Watts of reflected RF power at 2.45 GHz.

Surface analysis by X-ray photoelectron spectroscopy showed that amines (alkyl substituted) had been introduced to the molecular surface of the 7 micron pore size UHMWPE at levels on the order of 20 atom percent with little dependence on

10 sample temperature. In contrast, reflectance FTIR on identical specimens showed no detectable alkyl amine features. Together, the X-ray photoelectron and FTIR data show that the FDRC amination reaction is confined to the molecular surface of the porous polymer.

In addition, amine selective molecular probes such as fluorescein isothiocyanate (FITC) and fluorescein dichlorotriazine (DTAF) react with all

15 functionalized surfaces throughout the porous polyethylene article, while being unreactive with the unmodified surfaces. Cleavage of the isothiurea formed by reaction of FITC with alkyl amine functions with aqueous 0.1 M NaOH releases fluorescein into solution for direct spectrophotometric measurement and calculation of

20 the number of micromoles of amine function per gram of porous solid, which ranged between 0.2 and 0.5 micromoles of reactive amine per gram of the subject porous UHMWPE with 7 micron nominal pore size, after ammonia FDRC.

Additional measurements of pore surface alkyl amine content of 7-micron-pore-size porous UHMWPE after reaction under various conditions of time and

25 temperature in an ammonia FDRC environment like that described above resulted in surface amine concentrations of 0.4 micromoles per gram.

EXAMPLE 4

A sample of 7 micron pore size porous UHMWPE, of 0.25 inches in

30 thickness, was subjected to atomic oxygen FDRC conditions for at least one hour in a side-arm reactor as described above, with operating conditions similar to Example 3 except that aviators breathing oxygen was used as the working gas. No significant pressure rise was observed on plasma source ignition, as is characteristic of atomic oxygen FDRC.

35 Direct measurement of surface R-OH concentrations using the molecular probe D-1557 sulfonyl chloride from Molecular Probes Inc., Eugene Oregon, showed

0.03 micromoles of R-OH per gram sample, after base hydrolysis (which releases the chromophore) of the sulfonic acid ester formed by reaction of the D-1557 probe (1mg/ml in dry acetone with 1.0% pyridine) of the functionalized polymer surface.

5 A second identical sample of the functionalized porous UHMWPE was reacted with: 1) the D-1557 probe (1 mg/ml in dry acetone, 1% pyridine) followed by, 2) thorough washing with dry acetone and, 3) reaction with a 20 wt% solution of a 4 generation PAMAM Starburst™ dendrimer (amino terminated) for 3 days. The terminal amino groups on the dendrimer react with the sulfonic acid esters formed by reaction of D-1557 with the surface R-OH groups to produce D-NH-(dendrimer),
10 thereby immobilizing the dendrimer on the surfaces of the oxygen FDRC porous polymer. Subsequent analysis of the oxygen FDRC porous polymer using the FITC method of Example 3 above revealed that on the order of 0.2 micromoles per gram of primary amine function had been introduced by coupling the dendrimer to the surface.

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EXAMPLE 5

Starting with a sintered particle bed material having about 2,500 cm²/g surface area, 7 micron nominal pore size, and a density of about 0.65 g/cc such as Porex T³ filter material, and subjecting the subject starting material to FDRC ammonia or oxygen to produce 0.2 micromoles of R-OH or R-NH₂ per gram of the FDRC-T³, we
20 have, in 20 grams of FDRCT³, 4 micromoles of the FDRC functionality in a total volume of 30 cc with an 11 cc total pore volume. Given the pellicular structure of the MSPS ion exchange material, all 4 micromoles of capacity should be available to bind biomolecules such as protein. The reported higher capacity of some conventional resin beads, on the order of 4 millimoles per gram, corresponds to only about 15 to 45 mg of
25 protein per ml of packed bed or about 1 micromole of a 10,000 amu protein per ml of packed bed. Direct quaternization of surface R-NH₂ will produce an anion exchange media having a 4 micromole capacity in small, easily used package. However, reaction of the initial FDRC functionality (R-NH₂ or R-OH) with, for example trichloro-s-triazine (TST) in acetone with a pyridine catalyst or in cold aqueous bicarbonate at pH
30 8.5 produces R-NH-(N₃C₃Cl₂) or R-O-(N₃C₃Cl₂) allows further reaction to covalently immobilize soluble polymer chains, polymer latex microspheres or gels to greatly increase the ion exchange capacity of the resulting MSPS ion exchange material. Direct reaction of the remaining chlorines on the surface linked TST allows the creation of a variety of new MSPS ion exchange surface phases along with a substantial increase in
35 ion exchange capacity.

Direct reaction of the $R-NH-(N_3C_3Cl_2)$ or $R-O-(N_3C_3Cl_2)$ with polyethylene glycols (PEGs) of low molecular weight (such as are available commercially from Shearwater Polymers of Huntsville Alabama) which are hydroxy or amino terminated on one end and sulfonated or quartenized on the other results in a
5 tentacular stationary phase MSPS having a capacity of 8 micromoles in the twenty gram (30 cc) MSPS ion exchange article.

MSPS ion exchange capacity can be further increased by first reaction the $R-NH-(N_3C_3Cl_2)$ or $R-O-(N_3C_3Cl_2)$ with a trifunctional alkylamine such as TREN (Pressure Chemical Pittsburgh, Penn.) to multiply capacity by a factor of 4.
10 Functionalized PEGs can then be coupled, via terminal hydroxyls, to the primary amines of the surface immobilized TRENS using coupling reagents such as toluene sulfonyl chloride or other well known coupling reactions (E. Klien, 1991). The result is a tentacular stationary phase an ion exchange capacity of 16 micromoles in a twenty gram (30 cc) MSPS ion exchange article.

15 Direct attachment or graft polymerization of functionalized polymer chains such as polystyrene sulfonate, the quartenary ammonium form of polychloromethylstryene, polyacrylic acid, DEAE or other functionalized dextrans, increases the ion exchange capacity by 1,000 to 10,000 fold to produce a 4 to 40 millimole ion exchange capacity in a twenty gram (30 cc) MSPS ion exchange article.
20 Polymers may be coupled to form one of two distinct types of MSPS surface phase: 1) polymer brush phases in which the polymer chains are attached at one end only, and 2) lightly cross linked surface immobilized gel phases in which the polymers are covalently linked to the surface at any point along the linear or branched polymer chain and additional layers of polymer chains can also be immobilized by linking to a small
25 number of available sites (to maintain light cross linking) on the first layer of immobilized polymers. One again, a great variety of coupling reactions are available (E. Klein, 1991), for example, polymers with terminal repeat unit $R-OH$ or $R-NH_2$ groups can be linked to $R-NH-(N_3C_3Cl_2)$ or $R-O-(N_3C_3Cl_2)$ surfaces as described above for PEGs.

30 Surface graft polymerization can be achieved by either immobilization of a vinyl monomer or polymerization initiator of the FDRC activated resin, immersing the primed FDRC resin in a solution of the appropriate vinyl monomer solution and subjecting the solution to polymerizing conditions by, for example, elevating the temperature. For example, any amino surface MSPS can be used to immobilize an
35 AIBN type initiator such as 4,4'-azobis(4-cyanovaleric acid) using carbonyldimidazole coupling methods (E. Klien, 1991) or by prior conversion of the valeric acid functions

in the initiator to acid chlorides at low temperature followed by low temperature coupling to the amino MSPS substrate. The initiator derivitized MSPS can then be recovered, washed, and placed in a solution of appropriate vinyl monomers such as deinhibited vinyl acetate/acrylamide or vinyl pyrrolidone to produce a surface grafted polymer phase by elevating the temperature to induce vinyl polymerization.

A special class of polymers to immobilize on FDRC functionalized resins are the Starburst TM dendrimers, hyperbranched dendrimers, and fractal polymer phases. These unique stationary phases confer high degrees of surface functionalization while stabilizing the surface phase against the surface restructuring with polar group involution observed in some plasma treated polymers by extensively cross linking the surface. At the same time high degrees of surface functionalization are possible and unique stationary phases having controlled microporosity can be prepared. For example, amino terminated 4th generation Starburst TM dendrimers have been allowed to react with R-NH-(N₃C₃Cl) or R-O-(N₃C₃Cl) MSPS supports to produce MSPS ion exchange materials having up to 200 micromoles of amino function in 20 grams of the T³ Porex porous solid resin filter.

The performance of the new MSPS ion exchange article described above can be compared with prior art technology. Given 1500 mg of protein in 300l of lysis buffer, the total amount of stationary phase needed to retain all the protein assuming the usual 10 fold excess resin capacity is: 1) 600 ml of conventional resin bead column, 2) 150 Sartobind MA-100 membrane ion exchanger units or equivalent ion exchange area having a total void volume of 300 ml, or 3) 15 MSPS ion exchangers with 150 cc void volume. Clearly the MSPS article is able to produce the protein in a more concentrated final form. Both the MSPS and membrane systems can complete the separation in a much shorter time than in a packed resin bead column with the MSPS and membrane times being comparable. The additional performance advantages conferred by the tentacular type (PEG) ion exchange materials in the separation of biomolecules are available in some of the MSPS ion exchange materials.

30 EXAMPLE 6

This describes the FlowGen column design for large scale chromatography. There is an end housing which goes on both ends. The end housing surface which contacts the top or bottom of the cylindrical chromatography column has a labyrinthine design to permit better distribution of flow across the top of the reactor.

35 The end housing and the side housing can be made of simple materials, e.g., polyethylene or polypropylene, which can be snapped together to form a liquid tight

seal. There is no need for end frits or plungers or the like. The columns need no laborious gravity packing and are not subject to the channeling and bed cracking or bed packing problems of particulate columns. The are completely transparent to scale. The chromatography columns can be one piece or stacked discs or stacked columns, without the flow and scale restrictions of packed membranes.

EXAMPLE 7

Porex® X-4920 (Porex Technologies) was treated by remote plasma discharge using ammonia as the source gas, according to the general process described above, to create an amine-functionalized porous polymer according to the present invention. Coupling is observed by the retention of a blue color after extensive washing and in comparison to a control. A sample of blue-dyed 0.2 micron particle size poly(styrene-co-vinylamine) was coupled to the amine-functionalized Porex® X-4920 using trichloro-s-triazine as the coupling agent. The resulting novel structure of matter and its inventive method of preparation are aspects of the present invention. This novel structure provides a covalently-bound high-capacity supermolecular surface phase that may be used for biomolecule (*e.g.*, DNA) synthesis or chromatography.

EXAMPLE 8

According to the present invention, remote plasma discharge can be used to introduce functionality, *e.g.*, amine groups, to polystyrene, without the concomitant formation of benzylamine groups (which is a common byproduct that occurs during prior art processes wherein Friedel-Crafts alkylation provides chloromethylation of the aromatic rings, and those chloromethyl groups are reacted for form aminomethyl groups) The functionalized polystyrene of this invention can be used in various applications including chromatography and DNA synthesis.

Two hundred milligram samples of polystyrene beads (Pharmacia Biotech BA 5301, Batch 37, 29-5-97) were placed into each of two glass trays having a surface of about 4 inches by 6 inches. The polystyrene beads were distributed across the bottom of the glass trays, and then the trays were placed into a chamber where they were exposed to remote plasma according to the present invention. A source gas of 10% ammonia / 90% argon was used at a flow rate 685 sccm. The pressure inside the reaction chamber before activating the plasma was 4.805.13 torr. The plasma generator (ASTEX AX2000/DPC 25) was turned on to provide a radio frequency power at 2.4 GHz frequency of 100 Forward watts and 4 Reflected watts, and pressure inside the reactor increased by 0.33 torr to 5.13 torr. The polystyrene beads were exposed to the

amine radicals for 6 hours, followed by 20 minutes of contact with static source gas at 400 torr.

According to fluorescein isothiocyanate staining, the treated beads contained on the order of 100 μ mole amine groups/g of beads. A second titration procedure found that the beads contained 250 μ mole amine groups/g of beads, however this titration method did not consider the shift in pKa value due to the possible aromatic position of the NH groups. According to elemental analysis, the beads contained about 700 μ mole amine groups/g of beads.

These beads were reacted with nucleosides having 5'-trityl group hydroxyl protection and succinate esterification at the 3' hydroxyl. When reacted with the amine-functionalized polystyrene, an amide bond was formed, thereby covalently attaching the nucleoside to the solid support. The trityl group was then hydroxlyzed using DEBLOCK Acid (PerSeptive Biosystems) to yield a bright orange cation (the di(*p*-methylphenyl)phenylmethane cation). The orange cation concentration was measured spectroscopically at 498 nm to reveal a concentration of support-bound nucleosides of greater than about 12 μ mole /g of beads.

Thus, the present invention provides a composition comprising amine-functionalized polystyrene, with the proviso that no amine groups bonded to a primary carbon are present as part of the parent polystyrene structure.

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EXAMPLE 9

This Example illustrates the preparation and use of a representative 10-20 micron Porex™ microreactor within a diagnostic assay.

Porex™ porous solids are treated generally as described above without cleaning or pretreatment. The Porex™ materials are exposed to vacuum for not less than 1 hour prior to initiation of the atomic oxygen flow in the reactor. During exposure to the atomic oxygen flowing discharge, the Porex™ materials are supported by a perforated anodized aluminum treatment rack housed in an 11.4 cm inner diameter x 38 cm long horizontal Pyrex™ glass chamber. Reactive gas from the plasma generator enters the treatment chamber at the top, center of the cylinder so that gas flow is transverse to the long axis of the cylinder. This configuration produces fairly uniform oxygen atom concentrations over the middle third of the treatment rack.

An Astex S-250 microwave power supply is used to drive an Astex DPC25 plasma discharge head to produce the flowing discharge of atomic oxygen. The microwave power supply is operated at 250 watts forward RF power and <10 watts reflected RF power. The operating frequency is 2.4 GHz. Analyzed (MIL-0-27210 E)

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aviators breathing oxygen is used as the operating gas at a total system pressure of 2 Torr and a mass flow rate of 137 sccm. Pressure is measured with an MKS Baratron capacitance manometer and the mass flow rate of the oxygen gas is controlled with an MKS 1259C/type 247 mass flow controller system.

5 During treatments, oxygen atom concentration is measured and verified by weighing polyethylene thin film samples before and after the treatment. The absolute oxygen atom reaction efficiency of polyethylene films was determined, so that oxygen atom concentration and surface flux can be calculated directly from weight loss measurements on the thin films. Oxygen atom dose as a function of location is
10 determined using polyethylene film samples placed at intervals along the treatment rack.

 Treatment times of less than 4 hours produce complete treatments of the Porex™ materials. Complete treatment is verified by observing the uptake, by capillary action, of a 0.01% solution of the dye methylene blue in deionized water in Porex™
15 specimens placed in the reactor as quality assurance samples. The Porex™ are completely hydrophobic and will not take up the dye solution without treatment.

 Surface chemical characterization of the treated Porex™ is accomplished using wet chemical and instrumental methods.

 Samples (human sera, human cerebrospinal fluid, cell lysates and cell
20 culture media) and standard curves are then loaded onto the microreactor with a pipetman and incubated in 100% relative humidity at 37°C for 30 minutes. The microreactor is then washed and blotted 3-6 times. Antibody-enzyme conjugate detection reagent is then added and the microreactor is incubated at 37°C for 30 minutes. Again, the microreactor is washed and blotted 3-6 times.

25 The microreactor array is then placed in a 96 well reader plate, assay buffer is added and the microreactor is incubated for color development. The microreactor is then topped off with pusher buffer and the microreactor array fluid is driven into wells with mechanical pressure from a soft stopper array on a plate cover. The plate is then read in a standard plate reader.
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EXAMPLE 10

 Amino FlowRad™ macroporous polystyrene co-divinylbenzene Pharmacia (BAS 301, batch 31) beads (200 mg) were suspended in anhydrous dioxane (10 mL) and treated with carbonyldiimidazole (292 mg). The mixture was allowed to
35 stir at room temperature under a dry atmosphere for 24 hours. The mixture was then vacuum filtered and the beads were thoroughly washed with dioxane (2 x 10 mL),

acetonitrile (2 x 10 mL), and then with methylene chloride (2 x 10 mL). The activated imidazolyl urea intermediate (2; Figure 6) was then dried under high vacuum.

EXAMPLE 11

5 The activated imidazolyl urea intermediate (75 mg; 2 in Figure 6) was suspended in anhydrous toluene (5 mL) and anhydrous acetonitrile (5 mL). The mixture was treated with ethylenediamine (2 mL) and allowed to stir at room temperature for 24 hours under a dry atmosphere. The mixture was vacuum filtered and the beads were thoroughly washed with acetonitrile (10 mL), methanol (10 mL), and
10 methylene chloride (10 mL). The beads were dried under high vacuum for 16 hours. The beads were suspended in anhydrous toluene (2 mL), anhydrous pyridine (2 mL), and anhydrous N, N-dimethylformamide (DMF, 2 mL) and treated with 4-dimethylaminopyridine (DMAP, 10 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC, 95 mg), DMT-C^{Bz}-succinate (120 mg), and
15 triethylamine (TEA, 200 μ L). The mixture was allowed to stir at room temperature for 48 hours under a dry atmosphere. The mixture was vacuum filtered and washed and sonicated with 50% aqueous methanol, then with methanol, and finally with methylene chloride. Any unreacted sites of the material were then blocked by treating the beads with Cap A and Cap B (2 mL each, PerSeptive Biosystems) for 2 hours at room
20 temperature. The beads were vacuum filtered and thoroughly washed with acetonitrile, then with methanol, and finally with methylene chloride. The beads were dried under high vacuum for 16 hours. Determined loading: 13.43 μ mol/g

EXAMPLE 12

25 The activated imidazolyl urea intermediate (75 mg; 2 in Figure 6) was suspended in anhydrous toluene (5 mL) and anhydrous acetonitrile (5 mL). The mixture was treated with Jeffamine® XTJ-500 (2 mL) and stirred at room temperature for 24 hours under a dry atmosphere. The mixture was vacuum filtered and the beads were thoroughly washed with acetonitrile (10 mL), methanol (10 mL), and methylene
30 chloride (10 mL). The beads were dried under high vacuum for 16 hours and afforded material 3. The beads (3) were suspended in anhydrous toluene (2 mL), anhydrous pyridine (2 mL), and anhydrous DMF (2 mL) and treated with 4-dimethylaminopyridine (10 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95 mg), DMT-C^{Bz}-succinate (120 mg), and triethylamine (200 μ L). The
35 mixture was stirred at room temperature for 48 hours under a dry atmosphere. The mixture was vacuum filtered and washed and sonicated with 50% aqueous methanol,

then with methanol, and finally with methylene chloride. Any unreacted sites of the material were then blocked by treating the beads with Cap A and Cap B (2 mL each, PerSeptive Biosystems) for 2 hours at room temperature. The beads were vacuum filtered and thoroughly washed with acetonitrile, then with methanol, and finally with methylene chloride. The beads were dried under high vacuum for 16 hours. Determined loading: 3.44 $\mu\text{mol/g}$

EXAMPLE 13

The subject macroporous material with immobilized surface PEG linkers (200 mg; 3 in Figure 6) was suspended in DMF (20 mL) and tri-*n*-butylamine (300 mg). The mixture was cooled to 0°C and treated with iodomethane (400 mg). The mixture was allowed to warm to room temperature and stirred at room temperature for 16 hours. The mixture was vacuum filtered and washed thoroughly with DMF, then with acetonitrile, methanol, and finally with methylene chloride. The material was dried under high vacuum affording the quaternary iodide material. Silver nitrate (1.7g) was dissolved in water (17 mL) and heated to 85°C. Sodium hydroxide (390 mg in 17 mL water) was added and the mixture was stirred vigorously. Once coagulation of the silver oxide was complete, the mixture was decanted and the material was washed thoroughly with hot water. The quaternary iodide material (200 mg) was suspended in water (5 mL) and methanol (5 mL) and added to the damp silver oxide material. The mixture was stirred for 5 hours. The mixture was vacuum filtered and the material was thoroughly washed with hot water and dried to obtain the trimethylammonium hydroxide material.

EXAMPLE 14

The subject amino FlowRad™ macroporous beads (100 mg) were suspended in anhydrous N, N-dimethylformamide (10 mL) and acetonitrile (5 mL) and treated with *t*-Boc-NH-PEG-NHS (75 mg, Shearwater Polymers). The mixture was allowed to stir at room temperature for 24 hours. The mixture was vacuum filtered and the material was thoroughly washed with DMF, then acetonitrile, and finally with methylene chloride. The material was then treated with trifluoroacetic acid (5 mL) at room temperature for 6 hours. The mixture was vacuum filtered and the solid material was thoroughly washed and sonicated with methylene chloride and dried under high vacuum to afford material 4, as shown in Figure 6. Material 4 could be treated as in example 12 for the application of DNA synthesis or as in example 13 for use in anion exchange chromatography.

EXAMPLE 15

The subject amino FlowRad™ macroporous beads (300 mg; 1 in Figure 7) were suspended in acrylonitrile (10 mL). Glacial acetic acid (20 mmol per primary amino function) was added and the solution was heated under reflux for 24 hours. The mixture was allowed to cool and then vacuum filtered. The solid material was thoroughly washed with acetonitrile, then with methanol, and finally with methylene chloride. The nitrile intermediate that resulted from the acrylonitrile treatment was suspended in anhydrous tetrahydrofuran (THF, 5 mL) and treated with diisobutylaluminum hydride (5 mL of a 1M solution in THF). The mixture was allowed to stir at room temperature for 3 hours. The mixture was vacuum filtered and the solid material was suspended in THF (5 mL) and treated with ethyl acetate (5 mL). The mixture was vacuum filtered and the solid material was thoroughly washed with methylene chloride. The material was dried under high vacuum to afford 5, a material with 2n amino functions. This procedure may be repeated to obtain the desired amount of amplification of amino functions. The material can then be treated as in Example 12 for DNA synthesis or as in 13 for anion exchange chromatography.

EXAMPLE 16

The activated imidazolyl urea intermediate (2, 200 mg) was suspended in methanol (15 mL), toluene (5 mL), and dioxane (5 mL) and treated with Starburst PAMAM dendrimer generation 4.0. The mixture was allowed to stir at room temperature for 24 hours. The mixture was vacuum filtered and the material was washed thoroughly with methanol. The material was dried under high vacuum for 16 hours and afforded material 9. See Figure 8.

EXAMPLE 17

The macroporous dendrimer modified material (200 mg; 9 in Figure 8) was suspended in anhydrous toluene (10 mL) and anhydrous DMF (10 mL) and treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC, 120 mg), the appropriate DMT-nucleoside succinate (DMT-C^{Bz}-succinate, 45 mg), 4-dimethylaminopyridine (DMAP, 10 mg), and triethylamine (TEA, 25 μ L). The mixture was allowed to stir for 48 hours at room temperature under a dry atmosphere. The mixture was then vacuum filtered and the resin was washed and sonicated with 50% aqueous methanol (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The material was then treated with Cap A and Cap B (2 mL, each,

PerSeptive Biosystems) for 3 hours at room temperature (Cap A consists of acetic anhydride in tetrahydrofuran; Cap B consists of 1-methylimidazole in pyridine and tetrahydrofuran). The mixture was then vacuum filtered and the resin was washed thoroughly with acetonitrile (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The material was then dried under high vacuum for 16 hours and afforded material 10, $R = C^{Bz}$. Loading determinations were performed by treating a known amount of gel bead with a known volume of deblock solution (PerSeptive Biosystems, 2% dichloroacetic acid in methylene chloride). The orange color that was obtained due to the release of the dimethoxytrityl cation ($\epsilon_{503} = 76,000$) was then quantitated by UV spectrophotometry at 503 nm.

EXAMPLE 18

The macroporous material with immobilized surface dendrimers (200 mg; 9 in Figure 8) was suspended in DMF (20 mL) and tri-*n*-butylamine (300 mg). The mixture was cooled to 0°C and treated with iodomethane (400 mg). The mixture was allowed to warm to room temperature and allowed to stir at room temperature for 16 hours. The mixture was vacuum filtered and washed thoroughly with DMF, then with acetonitrile, methanol, and finally with methylene chloride. The material was dried under high vacuum affording the quaternary iodide material 11.

EXAMPLE 19

Silver nitrate (1.7g) was dissolved in water (17 mL) and heated to 85°C. Sodium hydroxide (390 mg in 17 mL water) was added and the mixture was stirred vigorously. Once coagulation of the silver oxide was complete, the mixture was decanted and the material was washed thoroughly with hot water. The quaternary iodide material (200 mg; 11 in Figure 8) was suspended in water (5 mL) and methanol (5 mL) and added to the damp silver oxide material. The mixture was stirred for 5h. The mixture was vacuum filtered and the material was thoroughly washed with hot water and dried to obtain the trimethylammonium hydroxide material 12.

EXAMPLE 20

The activated imidazolyl urea intermediate (200 mg; 2 in Figure 9) was suspended in DMF (15 mL), toluene (5 mL), and dioxane (5 mL) and treated with Star PEG (75 arms, weight average molecular weight (MW) 450,000). The mixture was allowed to stir at room temperature for 24 hours. The mixture was vacuum filtered and

the material was washed thoroughly with DMF, methanol, and methylene chloride. The material was dried under high vacuum for 16 hours and afforded material 14.

EXAMPLE 21

5 The macroporous material with immobilized surface Star PEGs (200 mg; 14 in Figure 9) was suspended in methylene chloride (25 mL) and treated with oxalyl chloride (500 μ L of a 2M solution in methylene chloride). The mixture was then treated with a catalytic amount of DMF and allowed to stir at room temperature for 24 hours. The mixture was vacuum filtered and washed thoroughly with methylene
10 chloride to afford material 15.

EXAMPLE 22

 The macroporous material with immobilized surface Star PEGs (200 mg; 14 in Figure 9) was suspended in anhydrous pyridine (25 mL) and treated with mesyl
15 chloride (500 mg). The mixture was allowed to stir at room temperature for 24 hours under a dry atmosphere. The mixture was vacuum filtered and the material was thoroughly washed with acetonitrile, then methanol, and finally with methylene chloride to afford material 16.

EXAMPLE 23

20 Material 15 or 16 (200 mg) was suspended in methylene chloride (20 mL) and treated with ammonia (5 mL of a 2M solution in methanol). See Figure 9. The mixture was allowed to stir at ambient temperature in a sealed vessel for 24 hours. The mixture was vacuum filtered and the material was thoroughly washed with
25 methanol and then with methylene chloride to afford material 17.

EXAMPLE 24

 The macroporous material with immobilized, aminated surface Star PEGs (200 mg; 17 in Figure 10) was suspended in anhydrous toluene (10 mL) and
30 anhydrous DMF (10 mL) and treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC, 120 mg), the appropriate DMT-nucleoside succinate (DMT-C⁸-succinate, 45 mg), 4-dimethylaminopyridine (DMAP, 10 mg), and triethylamine (TEA, 25 μ L). The mixture was allowed to stir for 48 hours at room temperature under a dry atmosphere. The mixture was then vacuum filtered and the
35 resin was washed and sonicated with 50% aqueous methanol (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The material was then

5 treated with Cap A and Cap B (2 mL, each, PerSeptive Biosystems) for 3 hours at room temperature (Cap A consists of acetic anhydride in tetrahydrofuran; Cap B consists of 1-methylimidazole in pyridine and tetrahydrofuran). The mixture was then vacuum filtered and the resin was washed thoroughly with acetonitrile (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The material was then dried under high vacuum for 16 hours and afforded material 10, $R = C^{Bz}$. Loading determinations were performed by treating a known amount of gel bead with a known volume of deblock solution (PerSeptive Biosystems, 2% dichloroacetic acid in methylene chloride). The orange color that was obtained due to the release of the dimethoxytrityl cation ($\epsilon_{503} = 76,000$) was then quantitated by UV spectrophotometry at 10 503 nm.

EXAMPLE 25

15 The macroporous material with immobilized, aminated surface Star PEGs (200 mg; 17 in Figure 10) was suspended in DMF (20 mL) and treated with tri-n-butylamine (300 mg). The mixture was cooled to 0°C and iodomethane (400 mg) was added dropwise over the course of 30 min. The mixture was allowed to warm to room temperature and allowed to stir at room temperature for 16 hours. The mixture was vacuum filtered and washed thoroughly with DMF, then with acetonitrile, methanol, 20 and finally with methylene chloride. The material was dried under high vacuum affording the quaternary iodide material 19.

EXAMPLE 26

Silver nitrate (1.7g) was dissolved in water (17 mL) and heated to 85°C. 25 Sodium hydroxide (390 mg in 17 mL water) was added and the mixture was stirred vigorously. Once coagulation of the silver oxide was complete, the mixture was decanted and the material was washed thoroughly with hot water. The quaternary iodide material (200 mg; 19 in Figure 10) was suspended in water (5 mL) and methanol (5 mL) and added to the damp silver oxide material. The mixture was stirred for 5h. 30 The mixture was vacuum filtered and the material was thoroughly washed with hot water and dried to obtain the trimethylammonium hydroxide material 20.

EXAMPLE 27

35 The polymer colloid particles used in this example are poly(50% styrene/49.8% chloromethylstyrene/0.2% divinylbenzene) and have a mean diameter of 0.46 microns (before solvent swelling) in a surfactant stabilized aqueous dispersion.

Attachment of these particles to the amino FlowRad™ Porex pore surfaces creates the pellicular monolith with a vast increase in useful functional group capacity per unit weight of Porex. The resulting structure has numerous performance advantages over the prior art materials.

5 Coupling a functionalized gel layer to the pore surfaces of a FlowRad™ activated macroporous solid using the polymer colloid approach results in a novel structure of matter (polymer colloid pellicular monolith, hereby dubbed nanolith) which has important performance advantages over the prior art. The surface gel layer has a much lower degree of cross linking than is possible when using gel particles which
10 must support their own weight and resist fluid flow pressures. Lower gel cross-linking produces better mass transfer rates and more usable space to accommodate synthesis of large molecules such as DNA. In addition, stand alone gel particles must have diameters on the order of 300 microns when swollen to produce useful packed beds which result in poor mass transfer performance as a result of the 150 micron diffusion
15 path to the center of the gel beads. The diffusion path in the FlowGenix polymer colloid pellicular monolith is less than 5 microns even if the polymer colloid particles attached to the amino FlowRad™ swell to 10 times the original colloidal dispersion diameter. Finally, the FlowGenix polymer colloid pellicular monolith can sustain higher fluid flow rates than commercial gel bead beds because the lightly cross linked
20 gel is mounted on a rigid Porex X-4920 support. It should be noted that the DNA synthesis support material reported here produces no fine particles that can clog fluid lines, valves or other fluid handling equipment.

Porex X-4920 (in the form of disks 6 mm in diameter and 1.6 mm in thickness) was thoroughly cleaned by using the following series of solvents in the order
25 presented followed by vacuum drying: 1) hexane, 2) N, N-dimethylformamide, 3) 2-propanol, 4) E-pure water, and 5) methanol. After vacuum out gassing for 16 h, the disks were subjected to amino FlowRad™ conditions for 6 h. The Porex disks were arrayed as a single layer in 4 inch by 6 inch glass sample trays in FlowGenix Pilot Reactor number 1 so that no UV light from the plasma source struck the Porex disks.
30 The Plasma was generated using an ASTEX AX 2000 microwave power supply driving an ASTEX DPC-25 plasma head. Pilot reactor operating parameters are summarized below:

Gas = 10% ammonia/90% argon, Aeriform LK-4375

Mass flow rate of gas = 398 sccm

35 Pressure plasma on = 3.25 Torr

Pressure plasma off = 3.02 Torr

Microwave power = 100-Watts forward/4-Watts reflected

Analysis of the amino FlowRad™-treated Porex X-4920 disks using the fluorescein isothiocyanate (FITC) method showed 0.06 micromoles of FITC reactive amine groups per gram of aminated Porex. Unmodified Porex X-4920 could not be loaded with a suitable deoxynucleoside succinate (due to a lack of functionalities). Amino FlowRad™ Porex X-4920 could be loaded with suitable deoxynucleoside succinates with loading determinations in the range of 0.045 to 0.075 $\mu\text{mol/g}$.

The amino FlowRad™ Porex was then used as the porous solid in the preparation of a polymer colloid pellicular monolith using a surfactant stabilized colloidal dispersion (10 wt % solids) of polymer microspheres. The polymer microspheres were 0.46 micron diameter beads composed of poly(50% styrene/49.8% chloromethylstyrene/0.2% divinylbenzene) which was purchased from Bangs Laboratories as a custom synthesis batch made to FlowGenix requirements (Bangs stock code P0004600BN). The Bangs microsphere dispersion (2 mL) was dialyzed in Spectra/Por 25000 MWCO dialysis tubing for 16 h against 50 mM sodium phosphate buffer (200 mL, pH 8.0) and then diluted 5-fold using fresh buffer. The diluted microsphere preparation (2 mL) was then added to the amino FlowRad™ Porex X-4920 (300 mg), sonicated for 2 min and placed in a 55°C incubator for 1 h. During this reaction period, surface amino groups in the amino FlowRad™ Porex displace the chloro substituents exposed on the chloromethylstyrene residues of the Bangs microspheres ($\text{R-NH}_2 + \text{R}'\text{-CH}_2\text{Cl} \Rightarrow \text{R-NH-CH}_2\text{-R}'$). At the end of the one hour incubation, the polymer colloid solution was aspirated off and the polymer colloid pellicular monolith disks were washed thoroughly, first three times with E-pure water and then three times with methanol. The pellicular monolith product was then prepared as described in the following examples. The variable degree of loadings that were determined illustrates the effect of the solvents on the efficiency of the chlorine displacement reaction.

COUNTER EXAMPLE 27.1

The amino FlowRad™ Porex polymer colloid (supplied in methanol) was treated with toluene (10 mL) and ammonia (10 mL of a 2M solution in methanol). The mixture was allowed to stir at room temperature for 24 h in a stoppered flask. The solvent was then decanted from the mixture and the plug discs were washed with methanol (2 x 5 mL), then with methylene chloride (5 mL) and then with dry DMF (N,N-dimethylformamide, 4 x 5 mL). The plugs were transferred to a dry DMF solution (2 mL) which contained anhydrous pyridine (2 mL) and anhydrous toluene (2

mL). The mixture was then treated with 4-dimethylaminopyridine (DMAP, 15 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC, 95 mg), triethylamine (TEA, 90 μ L), and a suitably protected deoxynucleoside succinate, DMT-C-(Bz)-succinate (45 mg). The mixture was allowed to stir at room temperature for 48 h under a dry atmosphere. The mixture was vacuum filtered and the discs were washed and sonicated with 50 % aqueous methanol, then methanol and finally with methylene chloride. The material was then treated with Cap A and Cap B (2 mL each) for 1.5 h at room temperature. The mixture was the vacuum filtered and washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 0.039 μ mol/g.

COUNTER EXAMPLE 27.2

The amino FlowRad™ Porex polymer colloid (supplied in methanol) was suspended in methanol (5 mL) and treated with ethylenediamine (1 mL). The mixture was allowed to stir at room temperature for 24 h in a stoppered flask. The solvent was then decanted from the mixture and the plug discs were washed with methanol (2 x 5 mL), then with methylene chloride (5 mL) and then with dry DMF (4 x 5 mL). The plugs were transferred to a dry DMF solution (2 mL) which contained anhydrous pyridine (2 mL) and anhydrous toluene (2 mL). The mixture was then treated with DMAP (15 mg), DEC (95 mg), TEA (90 μ L), and DMT-C-(Bz)-succinate (45 mg). The mixture was allowed to stir at room temperature for 48 h under a dry atmosphere. The mixture was vacuum filtered and the discs were washed and sonicated with 50 % aqueous methanol, then methanol and finally with methylene chloride. The material was then treated with Cap A and Cap B (2 mL each) for 1.5 h at room temperature. The mixture was the vacuum filtered and washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 0.142 μ mol/g.

COUNTER EXAMPLE 27.3

The amino FlowRad™ Porex polymer colloid (10 discs, supplied in methanol) was suspended in methanol (5 mL) and treated with Jeffamine® XTJ-500 (1 mL). The mixture was allowed to stir at room temperature for 24 h in a stoppered flask. The solvent was then decanted from the mixture and the plug discs were washed with methanol (2 x 5 mL), then with methylene chloride (5 mL) and then with dry DMF (4 x 5 mL). The plugs were transferred to a dry DMF solution (2 mL) which contained anhydrous pyridine (2 mL) and anhydrous toluene (2 mL). The mixture was then

5 treated with DMAP (15 mg), DEC (95 mg), TEA (90 μ L), and DMT-C-(Bz)-succinate (45 mg). The mixture was allowed to stir at room temperature for 48 h under a dry atmosphere. The mixture was vacuum filtered and the discs were washed and sonicated with 50 % aqueous methanol, then methanol and finally with methylene chloride. The material was then treated with Cap A and Cap B (2 mL each) for 1.5 h at room temperature. The mixture was the vacuum filtered and washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 0.076 μ mol/g.

COUNTER EXAMPLE 27.4

10 The amino FlowRad™ Porex polymer colloid (10 discs, supplied in methanol) was suspended in methanol (5 mL) and toluene (5 mL) and treated with Jeffamine® XTJ-500 (1 mL). The mixture was allowed to stir at room temperature for 24 h in a stoppered flask. The solvent was then decanted from the mixture and the plug discs were washed with methanol (2 x 5 mL), then with methylene chloride (5 mL) and
15 then with dry DMF (4 x 5 mL). The plugs were transferred to a dry DMF solution (2 mL) which contained anhydrous pyridine (2 mL) and anhydrous toluene (2 mL). The mixture was then treated with DMAP (15 mg), DEC (95 mg), TEA (90 μ L), and DMT-C-(Bz)-succinate (45 mg). The mixture was allowed to stir at room temperature for 48 h under a dry atmosphere. The mixture was vacuum filtered and the discs were washed
20 and sonicated with 50 % aqueous methanol, then methanol and finally with methylene chloride. The material was then treated with Cap A and Cap B (2 mL each) for 1.5 h at room temperature. The mixture was the vacuum filtered and washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 0.1328 μ mol/g.

PREFERRED EXAMPLE 27.5

25 The amino FlowRad™ Porex polymer colloid (supplied in methanol) was suspended in methanol (5 mL) and toluene (5 mL) and treated with ethylenediamine (1 mL). The mixture was allowed to stir at room temperature for 24 h in a stoppered flask. The solvent was then decanted from the mixture and the plug discs were washed with
30 methanol (2 x 5 mL), then with methylene chloride (5 mL) and then with dry DMF (4 x 5 mL). The plugs were transferred to a dry DMF solution (2 mL) which contained anhydrous pyridine (2 mL) and anhydrous toluene (2 mL). The mixture was then treated with DMAP (15 mg), DEC (95 mg), TEA (90 μ L), and DMT-C-(Bz)-succinate (45 mg). The mixture was allowed to stir at room temperature for 48 h under a dry

atmosphere. The mixture was vacuum filtered and the discs were washed and sonicated with 50 % aqueous methanol, then methanol and finally with methylene chloride. The material was then treated with Cap A and Cap B (2 mL each) for 1.5 h at room temperature. . The mixture was the vacuum filtered and washed and sonicated with
5 acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 14.68 $\mu\text{mol/g}$.

COUNTER EXAMPLE 27.6

The amino FlowRad™ Porex polymer colloid (4 discs, supplied dry) were suspended in methanol (5 mL) and toluene (5 mL) and treated with ammonia (2 mL of a
10 2M solution in methanol). The mixture was stirred at room temperature for 24 h in a sealed vessel. The mixture was vacuum filtered and the discs were washed and sonicated with methanol, then with acetonitrile and finally with methylene chloride. The discs were then dried under high vacuum for 16 h. The material was then treated with DMAP (10 mg), DEC (95 mg), TEA (100 μL), DMT-C(Bz)-succinate (45 mg),
15 anhydrous pyridine (2 mL), anhydrous toluene (2 mL), and anhydrous DMF (2 mL). The mixture was stirred at room temperature under a dry atmosphere for 48 h. The mixture was vacuum filtered and the discs were washed and sonicated with 50% aqueous methanol, then with methanol, and finally with methylene chloride. The discs were treated with Cap A and Cap B (2 mL each) for 2 h at room temperature. The
20 mixtures were vacuum filtered and the discs were washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 0.055 $\mu\text{mol/g}$.

COUNTER EXAMPLE 27.7

The amino FlowRad™ Porex polymer colloid (4 discs, supplied dry) were suspended in methanol (5 mL) and toluene (5 mL) and treated with ethylenediamine (1
25 mL). The mixture was stirred at room temperature for 24 h in a sealed vessel. The mixture was vacuum filtered and the discs were washed and sonicated with methanol, then with acetonitrile and finally with methylene chloride. The discs were then dried under high vacuum for 16 h. The material was then treated with DMAP (10 mg), DEC (95 mg), TEA (100 μL), DMT-C(Bz)-succinate (45 mg), anhydrous pyridine (2 mL),
30 anhydrous toluene (2 mL), and anhydrous DMF (2 mL). The mixture was stirred at room temperature under a dry atmosphere for 48 h. The mixture was vacuum filtered and the discs were washed and sonicated with 50% aqueous methanol, then with methanol, and finally with methylene chloride. The discs were treated with Cap A and

Cap B (2 mL each) for 2 h at room temperature. The mixtures were vacuum filtered and the discs were washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum. Determined loading: 0.024 $\mu\text{mol/g}$

5

COUNTER EXAMPLE 27.8

The amino FlowRad™ Porex polymer colloid (4 discs, supplied dry) were suspended in methanol (5 mL) and toluene (5 mL) and treated with Jeffamine® XTJ-500 (1 mL). The mixture was stirred at room temperature for 24 h in a sealed vessel. The mixture was vacuum filtered and the discs were washed and sonicated with
10 methanol, then with acetonitrile and finally with methylene chloride. The discs were then dried under high vacuum for 16 h. The material was then treated with DMAP (10 mg), DEC (95 mg), TEA (100 μL), DMT-C(Bz)-succinate (45 mg), anhydrous pyridine (2 mL), anhydrous toluene (2 mL), and anhydrous DMF (2 mL). The mixture was stirred at room temperature under a dry atmosphere for 48 h. The mixture was vacuum
15 filtered and the discs were washed and sonicated with 50% aqueous methanol, then with methanol, and finally with methylene chloride. The discs were treated with Cap A and Cap B (2 mL each) for 2 h at room temperature. The mixtures were vacuum filtered and the discs were washed and sonicated with acetonitrile, then with methanol, and finally with methylene chloride. The material was then dried under high vacuum.
20 Determined loading: 0.094 $\mu\text{mol/g}$

PREFERRED EXAMPLE 27.9

Excess solvent from the amino FlowRad™ Porex polymer colloid (4 discs, 100 mg, supplied in methanol) was wicked on a paper towel and washed thoroughly with toluene (4 x 5 mL). The material was again placed on a paper towel to
25 wick away excess solvent and suspended in dry toluene (5 mL) and dry DMF (5 mL) and ethylenediamine (1 mL). The mixture was allowed to stir at room temperature for 24 h under a dry atmosphere. The solvent was decanted from the mixture and the discs were washed with toluene (3 x 5 mL). The discs were then suspended in dry toluene (2 mL), dry DMF (2 mL), and dry pyridine (2 mL) and treated with DMAP (5 mg), DEC
30 (40 mg), TEA (25 μL), and DMT-C-Bz-succinate (18 mg). The mixture was allowed to stir at room temperature under a dry atmosphere for 72 h. The mixture was vacuum filtered and the discs were washed and sonicated with 50% aqueous methanol (10 mL) then with methanol (10 mL) and finally with methylene chloride (10 mL). The discs were then treated with Cap A and Cap B (1 mL each) for 2 h at room temperature.

Again, the mixture was vacuum filtered and the discs were washed with acetonitrile (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The discs were dried under high vacuum. Determined loading: 10.78 $\mu\text{mol/g}$.

PREFERRED EXAMPLE 27.10

5 Excess solvent from the amino FlowRad™ Porex polymer colloid (5 discs, 125 mg, supplied in methanol) was wicked on a paper towel and washed thoroughly with toluene (4 x 5 mL). The material was again placed on a paper towel to wick away excess solvent and suspended in dry toluene (5 mL) and dry DMF (5 mL) and treated with Jeffamine® XTJ 504 (1 mL). The mixture was allowed to stir at room
10 temperature for 24 h under a dry atmosphere. The solvent was decanted from the mixture and the discs were washed with toluene (3 x 5 mL). The discs were then suspended in dry toluene (2 mL), dry DMF (2 mL), and dry pyridine (2 mL) and treated with DMAP (5 mg), DEC (40 mg), TEA (25 μL), and DMT-C-Bz-succinate (18 mg). The mixture was allowed to stir at room temperature under a dry atmosphere for 72 h.
15 The mixture was vacuum filtered and the discs were washed and sonicated with 50% aqueous methanol (10 mL) then with methanol (10 mL) and finally with methylene chloride (10 mL). The discs were then treated with Cap A and Cap B (1 mL each) for 2 h at room temperature. Again, the mixture was vacuum filtered and the discs were washed with acetonitrile (10 mL), then with methanol (10 mL), and finally with
20 methylene chloride (10 mL). The discs were dried under high vacuum. Determined loading: 13.74 $\mu\text{mol/g}$.

PREFERRED EXAMPLE 27.11

 Excess solvent from the amino FlowRad™ Porex polymer colloid (5 discs, 125 mg, supplied in methanol) was wicked on a paper towel and washed
25 thoroughly with toluene (4 x 5 mL). The material was again placed on a paper towel to wick away excess solvent and suspended in dry toluene (5 mL) and dry DMF (5 mL) and treated with triethylamine (1 mL) and Jeffamine® XTJ 504 (1 mL). The mixture was allowed to stir at room temperature for 24 h under a dry atmosphere. The solvent was decanted from the mixture and the discs were washed with toluene (3 x 5 mL). The
30 discs were then suspended in dry toluene (2 mL), dry DMF (2 mL), and dry pyridine (2 mL) and treated with DMAP (5 mg), DEC (40 mg), TEA (25 μL), and DMT-C-Bz-succinate (18 mg). The mixture was allowed to stir at room temperature under a dry atmosphere for 72 h. The mixture was vacuum filtered and the discs were washed and sonicated with 50% aqueous methanol (10 mL) then with methanol (10 mL) and finally

with methylene chloride (10 mL). The discs were then treated with Cap A and Cap B (1 mL each) for 2 h at room temperature. Again, the mixture was vacuum filtered and the discs were washed with acetonitrile (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The discs were dried under high vacuum.

5 Determined loading: 13.64 $\mu\text{mol/g}$.

EXAMPLE 28

Excess solvent from the amino FlowRad™ Porex polymer colloid (5 discs, 125 mg, supplied in methanol) was wicked on a paper towel and the discs were
10 suspended in an aqueous solution of poly(allylamine) (20 mL, pH 8.5). The mixture was allowed to stir at room temperature for 24 h. The solution was decanted from the mixture and the discs were washed thoroughly with water, then with methanol, and finally with toluene (3 x 5 mL). The discs were then suspended in dry toluene (2 mL), dry DMF (2 mL), and dry pyridine (2 mL) and treated with DMAP (5 mg), DEC (40
15 mg), TEA (25 μL), and DMT-C-Bz-succinate (18 mg). The mixture was allowed to stir at room temperature under a dry atmosphere for 72 h. The mixture was vacuum filtered and the discs were washed and sonicated with 50% aqueous methanol (10 mL) then with methanol (10 mL) and finally with methylene chloride (10 mL). The discs were then treated with Cap A and Cap B (1 mL each) for 2 h at room temperature. Again, the
20 mixture was vacuum filtered and the discs were washed with acetonitrile (10 mL), then with methanol (10 mL), and finally with methylene chloride (10 mL). The discs were dried under high vacuum.

EXAMPLE 29

25 ArgoGel (NH_2 , 300 mg, Argonaut Technologies) was loaded with DMT- C^{Bz} succinate (75 mg) in the same manner as the above examples using DMAP (10 mg), DEC (100 mg) and triethylamine (100 μL) in anhydrous toluene (10 mL) and anhydrous DMF (5 mL). The work-up and capping of unreacted sites was the same as the examples above. Determined loading for ArgoGel: 116.88 $\mu\text{mol/g}$.

30 TentaGel (S-NH_2 , 80-100 μm , 300 mg, Advanced Chemtech) was loaded with DMT- C^{Bz} succinate (75 mg) in the same manner as the above examples using DMAP (10 mg), DEC (100 mg) and triethylamine (100 μL) in anhydrous toluene (10 mL) and anhydrous DMF (5 mL). The work-up and capping of unreacted sites was the same as the examples above. Determined loading for TentaGel: 45.84 $\mu\text{mol/g}$.

EXPERIMENTAL

A 0.2 μ mol scale synthesis of a desired oligonucleotide sequence was performed on a PerSeptive Biosystems Expedite 8900 Nucleic Acid Synthesis System using phosphoramidite chemistry. All necessary reagents for the Expedite synthesizer were purchased from PerSeptive Biosystems. The oligonucleotides were cleaved from the pellicular monolith polymer colloid and deprotected with ammonium hydroxide at 65°C for 4 h. Purification of the oligonucleotides was performed on a Pharmacia NAP-25 sephadex column. The oligonucleotides were analyzed by electrophoresis (20% Bis acrylamide) and by densitometry. The purity of the oligonucleotides was further analyzed by anion exchange HPLC. Anion exchange HPLC was performed on a Dionex HPLC system equipped with a Mono Q HR 5/5 column (Pharmacia Biotech). The oligonucleotides were eluted with 20 mM Tris-HCl (pH 8.0) using a sodium chloride gradient. The sequence of the 15 base long oligonucleotide: 5'-TCT AGC TAG CTA GCC-3'. A random sequence was used in the preparation of the 75- and 200-base long oligonucleotides.

OLIGONUCLEOTIDE SYNTHESIS RESULTS

The material described in examples 27.9 (oligonucleotide 16-61), 27.10 (oligonucleotide 16-63) and 27.11 (oligonucleotide 16-62) was used to prepare the 15-base long sequence of DNA. The quality of the DNA prepared on these supports is illustrated on an acrylamide gel (Figure 11) and on an HPLC chromatogram (Figure 12). The DNA prepared on the material described in example 27.10 (oligonucleotide 16-86) was also compared to DNA prepared on TentaGel (oligonucleotide 16-87), ArgoGel (oligonucleotide 16-88), and CPG (500Å, oligonucleotide 16-89), (Figures 13 and 14). Furthermore, the trityl records (which indicate coupling efficiency) in Figures 15, 16A, and 16B illustrate the performance of the nanolith material (example 27.10) for preparing long oligomers (75-bases long and 200-bases long, respectively). The trityl record (coupling efficiency) for the synthesis of the 200-base long oligonucleotide on 1000Å CPG is illustrated in Figures 17A and 17B for comparison.

TentaGel consists of terminally functionalized polyethylene glycols grafted onto polystyrene (1% divinylbenzene). The combination of non-polar polystyrene and polar polyethylene glycol results in a resin that swells in many solvents including certain solvents that would not ordinarily swell plain polystyrene beads. Like TentaGel, ArgoGel consists of terminally functionalized polyethylene glycols grafted onto polystyrene (1% divinylbenzene). The fundamental difference between ArgoGel and TentaGel is the number of polyethylene glycol units per styrene residue. Our

Nanolith material (the material prepared in example 27.10) demonstrates advantages over ArgoGel and TentaGel in the preparation of short oligomers. Substantial amounts of failure sequences were found to occur with ArgoGel and TentaGel. For the preparation of long oligomers (75-mer and 200-mer), our nanolith material showed superior performance to CPG (1000Å).

TABLE 4

PERFORMANCE COMPARISON OF NANOLITH AND OTHER SOLID PHASE SUPPORTS IN THE PREPARATION OF A 15-BASE LONG OLIGONUCLEOTIDE

Material	Final OD (units)	Final Yield (%)	Purity- Densitometry	Purity-HPLC
Nanolith	16.1	52.2	88.3	68.1
TentaGel	7.9	25.9	29.1	27.7
ArgoGel	17.6	57.8	8.4	6.7
CPG (500 Å)	17.1	55.5	90.7	67.8

TABLE 5

PERFORMANCE COMPARISON OF NANOLITH AND 1000Å CPG FOR LONG OLIGOMERS

Material (Oligonucleotide length)	Final OD (units)	Final Yield (%)
Nanolith (75-mer)	42.7	26.8
Nanolith (200-mer)	55.9	13.1
CPG-1000 Å (200-mer)	89.5	20.9

15

The invention provides new types of solid-phase supports for the separation and purification of organic and biochemicals by adsorptive, absorptive and chromatographic processes. The present invention permits the realization of novel, useful and practical separation media which cannot be achieved using the methods of

20

the prior art. The invention also provides new types of solid phase supports for chemical syntheses, and for assays used to diagnose or to monitor disease.

5 While the invention has been described in terms of specific embodiments, it is evident in view of the foregoing description that numerous alternatives, modifications and variations will be apparent to those skilled in the art. Thus, the invention is intended to encompass all such alternatives, modifications and variations which fall within the scope and spirit of the invention and the appended claims.

CLAIMS

What is claimed is:

1. A porous article having an external surface, a bulk matrix and pores extending from the external surface into the bulk matrix, wherein the pores define a pore surface, wherein the bulk matrix is formed, at least in part, of an organic polymer comprising carbon and hydrogen atoms, and the external and pore surfaces are formed, at least in part, of the same organic polymer having some of the hydrogen atoms replaced with moieties selected from the group consisting of nitrogen atom, oxygen atom, amino group, hydroxyl group, carbonyl group, and carboxylic acid group, and wherein the external surface does not display surface roughness, according to SEM analysis, due to ablation of surface carbon atoms or chain scission.
2. A porous solid stationary phase for liquid chromatography, comprising the porous article of claim 1 wherein the organic polymer has some of the hydrogen atoms replaced with functional groups selected from group consisting of amino, hydroxyl, carbonyl, and carboxylic acid.
3. The porous solid phase stationary phase for liquid chromatography of claim 2 wherein the amino, hydroxyl, carbonyl or carboxylic acid functional group is covalently linked, either directly or through a spacer group, to a chromatographically active group.
4. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to an anion exchanger.
5. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to a cation exchanger.
6. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to a hydrophobic group.
7. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to a hydrazide group.

8. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to a reactive group for covalent bond formation through protein amino groups.
9. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to a substituent group for reversed phase chromatography.
10. The porous solid stationary phase for liquid chromatography of claim 3 wherein the functional groups are linked to a substituent group for chiral chromatography.
11. The porous solid stationary phase for liquid chromatography of claims 3-9 containing a spacer group.
12. The porous solid stationary phase for liquid chromatography of claim 11 wherein the spacer group is selected from a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, a pellicular phase and a fractal polymer phase.
13. The porous solid stationary phase for liquid chromatography of claim 11 wherein the spacer group is a grafted polymer having a plurality of amine or hydroxyl groups.
14. A device for liquid chromatography comprising:
 - a housing, comprising
 - a liquid inlet, a wall member, and a liquid outlet;
 - having said liquid inlet in liquid communication with;
 - a porous solid stationary phase having an external surface, a bulk matrix and pores extending from the external surface into the bulk matrix, wherein the pores define a pore surface, wherein the bulk matrix is formed, at least in part, of an organic polymer comprising carbon and hydrogen atoms, and the external and pore surfaces are formed, at least in part, of the same organic polymer having some of the hydrogen atoms replaced with functional groups selected from amino, hydroxyl, carbonyl, and carboxylic acid, and wherein the external surface does not display surface roughness due to ablation of surface carbon atoms or chain scission;
 - and having said wall member in substantially continuous contact with the external surface of said solid stationary phase and affixed thereto in a manner requiring that

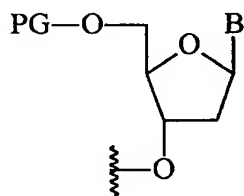
substantially all liquid flow proceeds axially through the solid stationary phase and substantially no liquid flow proceeds between said solid stationary phase and said wall member; and

having said liquid outlet in liquid communication with said solid stationary phase and positioned substantially axially opposite said liquid inlet.

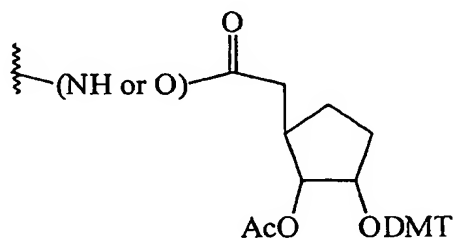
15. The porous article of claim 1 wherein the organic polymer has some of the hydrogen atoms are replaced with either an oxygen atom or a nitrogen atom, and wherein the oxygen atom or nitrogen atom is bonded, either directly or indirectly, to a protected nucleoside or amino acid.

16. The porous article of claim 15, comprising sintered non-porous polyolefin particles.

17. The porous article of claim 15 wherein the nitrogen atom or oxygen atom is directly or indirectly bonded to a protected nucleoside of the formula



18. The porous article of claim 15 wherein the nitrogen atom or oxygen atom is directly or indirectly bonded to a universal support of the formula



19. The porous article of claim 15 wherein a spacer moiety is intermediate the nitrogen or oxygen atom and the protected nucleoside or amino acid.

20. The article of claim 19 wherein the spacer moiety is a surface polymer brush phase, a lightly crosslinked polymer phase, a dendrimer phase, or a fractal polymer phase.

21. The article of claim 19 wherein the spacer moiety is a grafted polymer having a plurality of amine or hydroxyl groups.

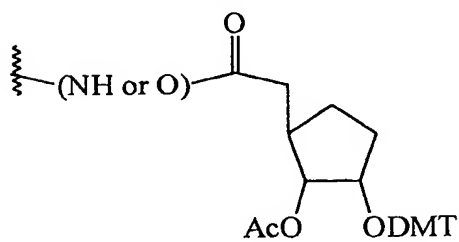
22. The porous article of claim 15, having a void volume of from about 35% to 60%.

23. The porous article of claim 15, having an effective pore diameter of about 0.01 microns to 2000 microns.

24. The porous article of claim 15, in the form of a disc or a monolith.

25. The porous article of claim 15, having surface functionality of at least 10^{-12} moles/cm².

26. A universal support for solid phase synthesis, having the structure



AcO ODMT, wherein the solid support is an insoluble organic or inorganic material which is inert to the reaction conditions employed for the solid phase synthesis.

27. The universal support of claim 26 wherein the solid support is selected from controlled pore glass beads, polystyrene beads, polystyrene/divinylbenzene beads, polystyrene/polyethylene glycol beads, polymeric membranes, polymeric films, and sintered polymeric particles.

28. A device for solid phase synthesis, comprising a porous article of claim 15 positioned within a housing.

29. The device of claim 28 wherein the porous article is in the form of a monolith or a plurality of discs.

30. The device of claim 28 which does not contain particles or powder.

31. A device for solid phase synthesis comprising a universal support of claim 26 positioned within a housing.

32. The device of claim 31 wherein the porous article is in the form of a monolith or a plurality of discs.

33. The device of claim 31 which does not contain particles or powder.

34. A method for detecting a target molecule in a sample, comprising the steps of:

(a) combining a sample with a diagnostic agent having affinity for a target molecule and immobilized on a porous article of claim 1, under conditions and for a time sufficient to allow the target molecule to bind to the diagnostic agent, wherein the organic polymer has some of the hydrogen atoms replaced with functional groups selected from group consisting of amino, hydroxyl, carbonyl, and carboxylic acid, and

(b) detecting the target molecule bound to the diagnostic agent, and therefrom detecting the presence of the target molecule in the sample.

35. A method according to claim 34 wherein the surface-functionalized porous article has a pore volume that is within 10% of the pore volume prior to surface functionalization.

36. A method according to claim 34 wherein said diagnostic agent is selected from the group consisting of antigens, antibodies, polynucleotides, enzymes, ligands and receptors.

37. A method according to claim 34 wherein said sample is obtained from a patient.

38. A method according to claim 37 wherein said sample is selected from the group consisting of blood, serum, urine, saliva, sputum, bronchial aspirates, breast duct aspirates, feces, cervical secretions, synovial and cerebrospinal fluid, intestinal irrigate, gastric fluid and tissue samples.

39. A method according to claim 34 wherein the organic polymer is a polyethylene, and wherein the porous article is modified to comprise hydroxyl groups on the exterior and interstitial surfaces.

40. A method according to claim 34 wherein the organic polymer is a polyethylene, and wherein the porous article is modified to comprise amino groups on the exterior and interstitial surfaces.

41. A method according to claim 34 wherein the article is in the form of a membrane.

42. A method according to claim 34 wherein the article is in the form of a particle.

43. A method according to claim 34 wherein the diagnostic agent is covalently bound to the article.

44. A method according to claim 34 wherein the diagnostic agent is noncovalently immobilized on the article.

45. A method according to claim 34 wherein the diagnostic agent has affinity for a target molecule selected from the group consisting of ubiquitin and prostate-specific antigen.

46. A method according to claim 34 wherein the step of detecting comprises a competitive binding assay.

47. A method according to claim 34 wherein the step of detecting comprises an immunoassay.

48. A method according to claim 34 wherein the step of detecting is radiometric.

49. A method according to claim 34 wherein the step of detecting is fluorometric.

50. A method according to claim 34 wherein the step of detecting is spectrophotometric.

51. A method according to claim 34 wherein the target molecule is selected from the group consisting of antigens, antibodies, polynucleotides, enzymes, substrates, ligands and receptors.

52. A kit for detecting a target molecule in a sample, comprising:

(a) a diagnostic agent having affinity for a target molecule and immobilized on a surface-functionalized porous article,

wherein the porous article comprises at least one organic polymer and has (1) a bulk matrix, (2) an exterior surface and (3) pores extending from the exterior surface into the bulk matrix and defining an interstitial surface,

wherein some hydrogen atoms present within the organic polymer of exterior and interstitial surfaces are replaced with one or more functional groups selected from the group consisting of amino, hydroxyl, carbonyl and carboxylic acid groups, and

wherein the exterior surface does not display appreciable surface roughness, according to SEM analysis, relative to the surface of an unmodified articles; and

(b) a detection reagent.

53. A kit according to claim 52 wherein the surface-functionalized porous article has a pore volume that is within 10% of the pore volume prior to surface functionalization.

54. A microreactor array assay system, comprising a two or a three dimensional array of porous solid microreactors,

wherein each microreactor comprises a surface-functionalized porous article;

wherein each porous article comprises at least one organic polymer and has (1) a bulk matrix, (2) an exterior surface and (3) pores extending from the exterior surface into the bulk matrix and defining an interstitial surface,

wherein some hydrogen atoms present within the organic polymer of exterior and interstitial surfaces are replaced with one or more functional groups selected from the group consisting of amino, hydroxyl, carbonyl and carboxylic acid groups, and

wherein the exterior surface does not display appreciable surface roughness, according to SEM analysis, relative to the surface of an unmodified articles;

wherein at least one two-dimensional array comprises a diagnostic agent immobilized on the surface of a surface-functionalized porous article;

said porous solid microreactors being capable of receiving sample and reagent liquids and taking up said sample and reagent liquids by capillary action combined with gravity flow; and

each of said porous microreactors being separated from said other porous solid microreactors in an x-dimension and a y-dimension such that cross-talk between said porous solid microreactors is substantially eliminated.

55. A microreactor array assay system according to claim 54 wherein the surface-functionalized porous article has a pore volume that is within 10% of the pore volume prior to surface functionalization.

56. A microreactor array assay system according to claim 54 wherein said porous solid microreactors are in register and in contact with one another in a z-dimension; whereby flow from one porous solid microreactor to the next porous solid microreactor results when one two dimensional porous solid microreactor array is brought into contact with another porous solid microreactor array.

57. The microreactor reactor array assay system according to claim 54, further comprising an absorbent towel or sheet for drawing fluid out of the porous solid microreactor array.

58. A method for detecting a target molecule in a sample, comprising:

(a) combining a sample with a diagnostic agent having affinity for a target molecule and immobilized on a porous article contained within a microreactor array assay system according to claim 54, under conditions and for a time sufficient to allow the target molecule to bind to the diagnostic agent; and

(b) detecting the target molecule bound to the diagnostic agent, and therefrom detecting the presence of the target molecule in the sample.

59. A method according to claim 58, wherein the step of detecting comprises an immunoassay.

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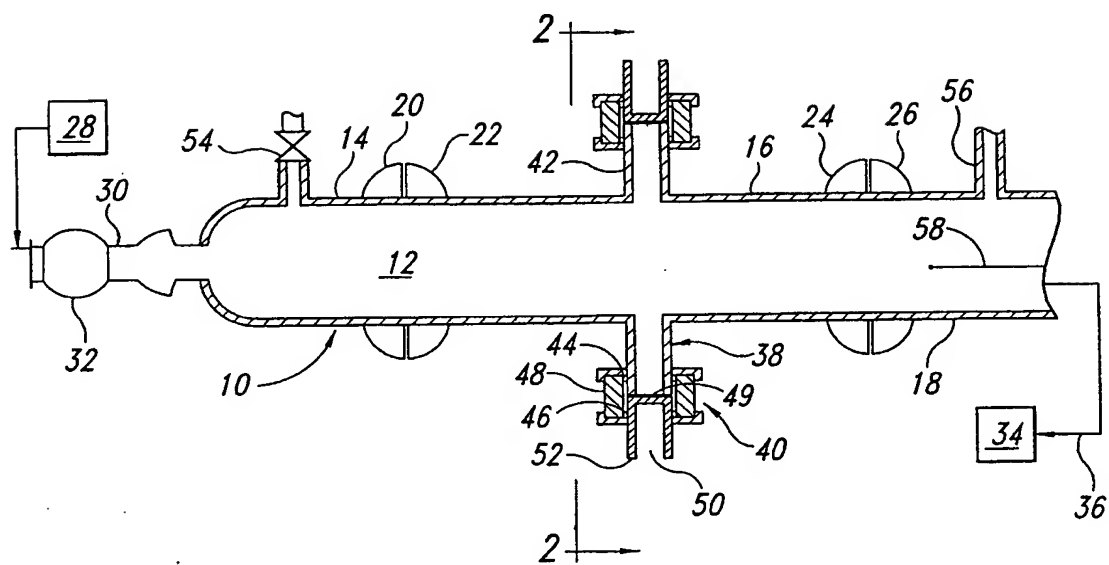


Fig. 1

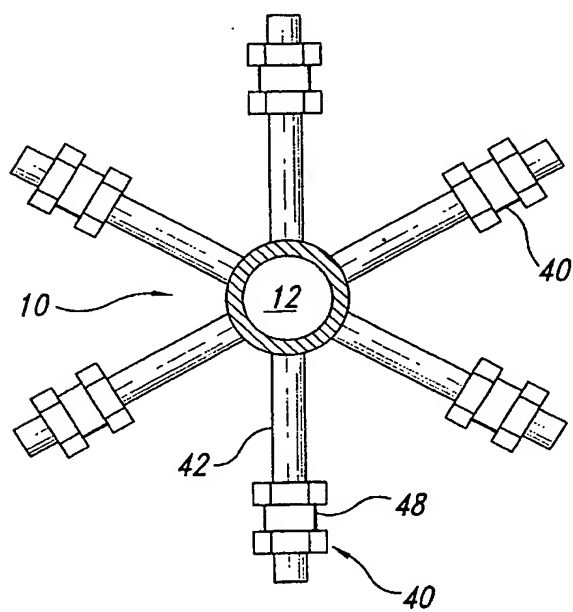
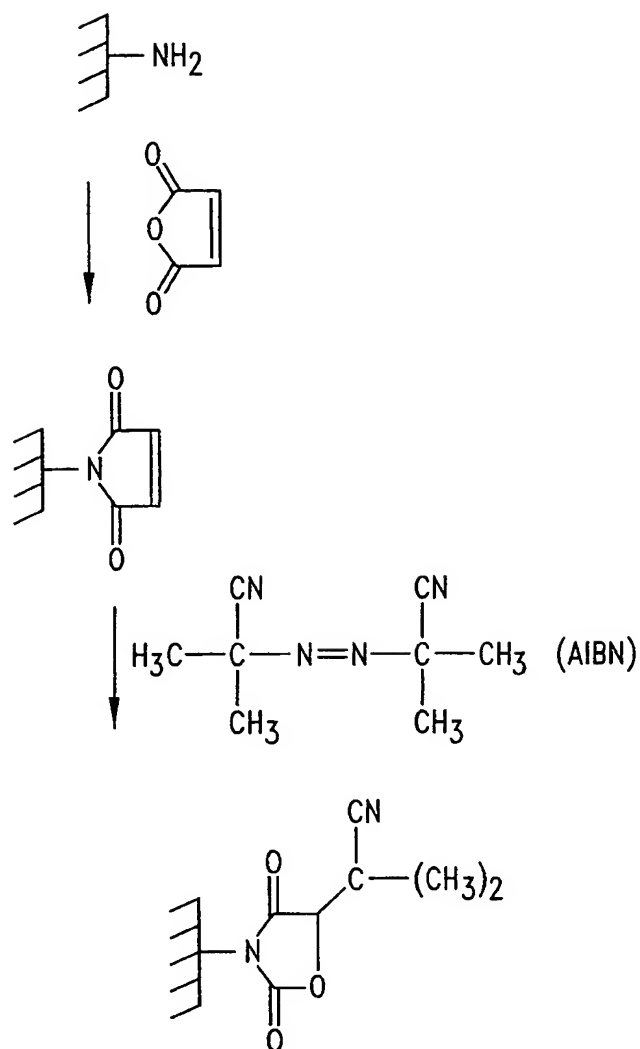


Fig. 2

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*Fig. 3A*

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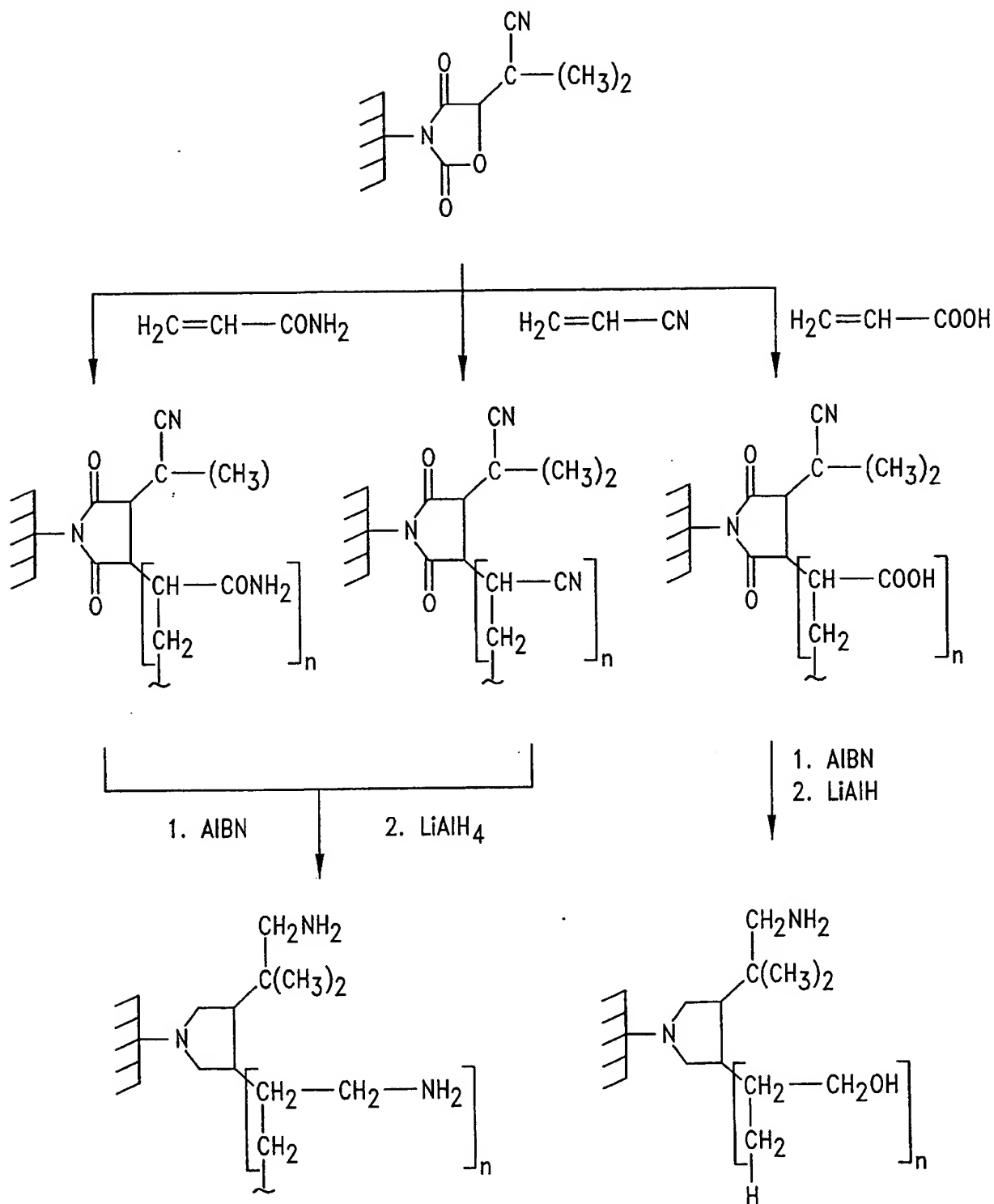


Fig. 3B

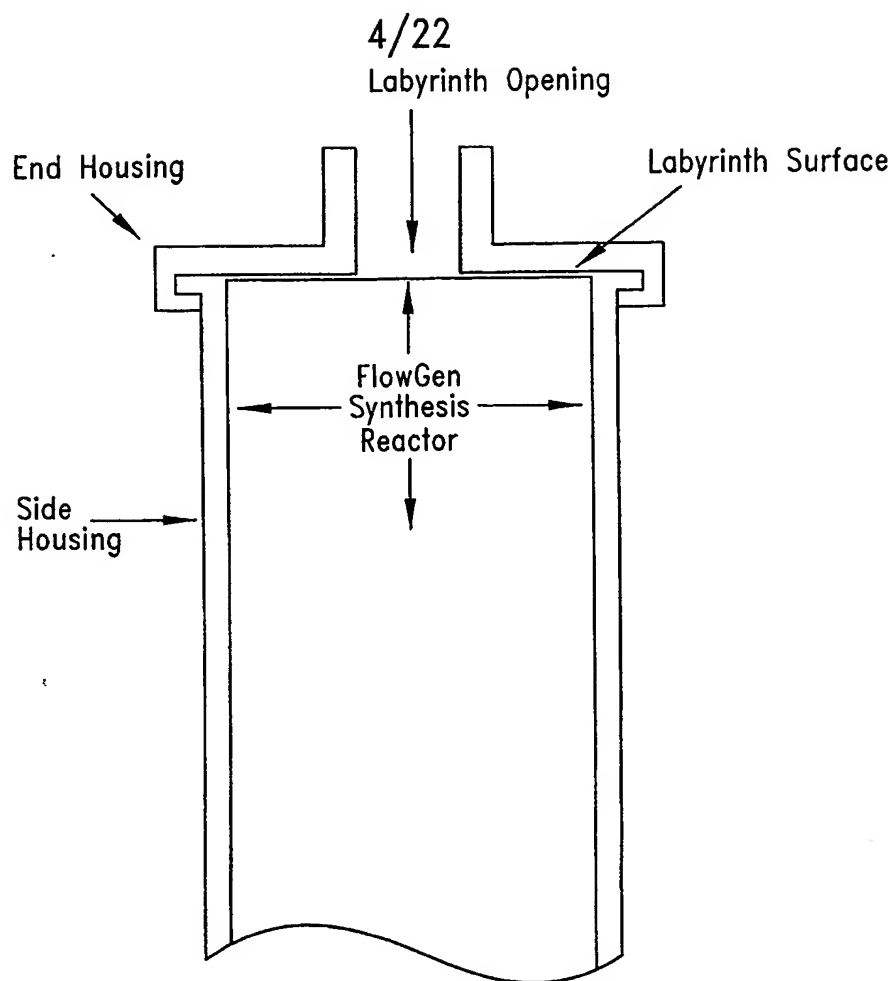


Fig. 4A

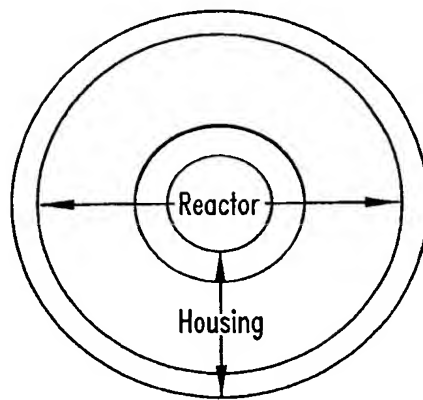


Fig. 4B

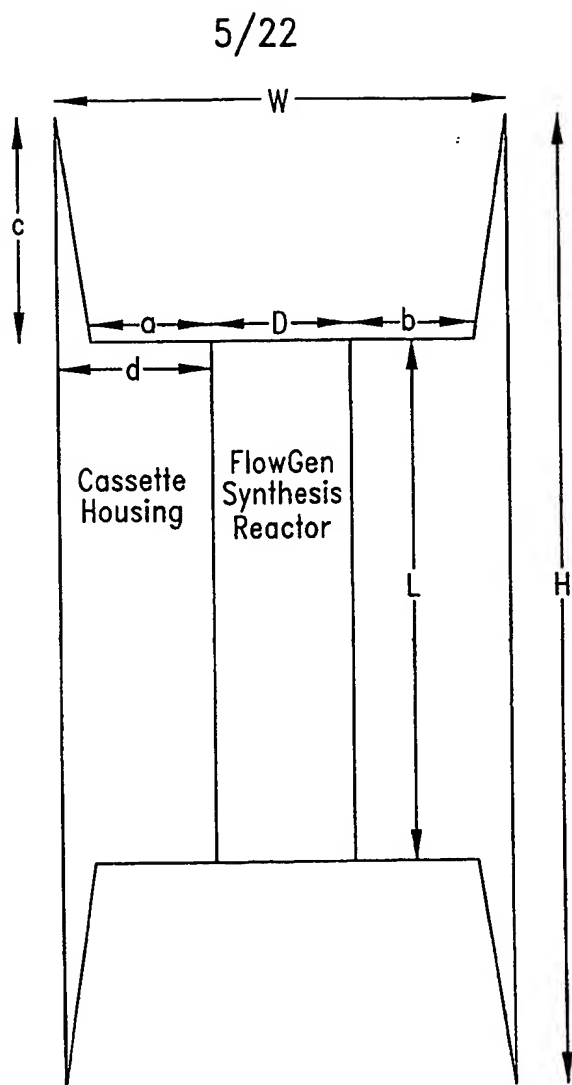


Fig. 5A

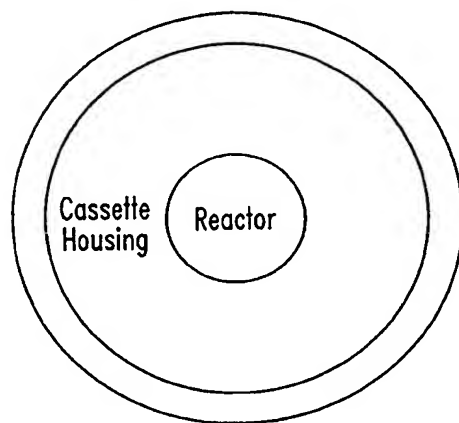
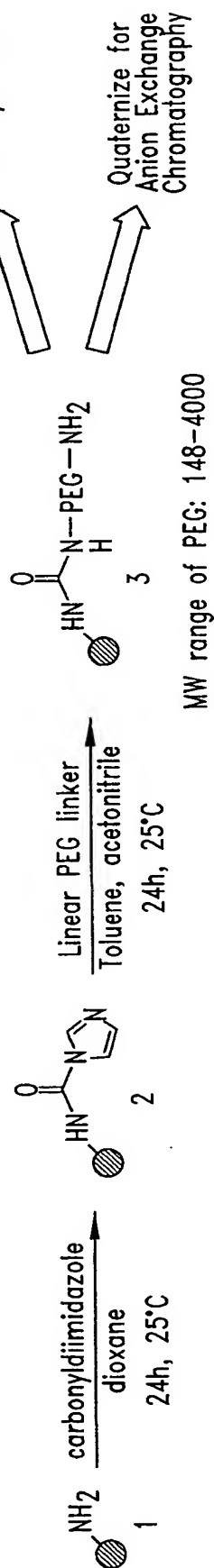


Fig. 5B

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Modification of AFR-Treated materials with Linear PEGs for Use in DNA Synthesis and Anion Exchange Chromatography

Coupling of Industrial PEG Linker Arm to AFR-Treated Materials



NH_2 = AFR-Treated Material

Coupling of Well-Characterized PEG Linker Arm to AFR-Treated Materials

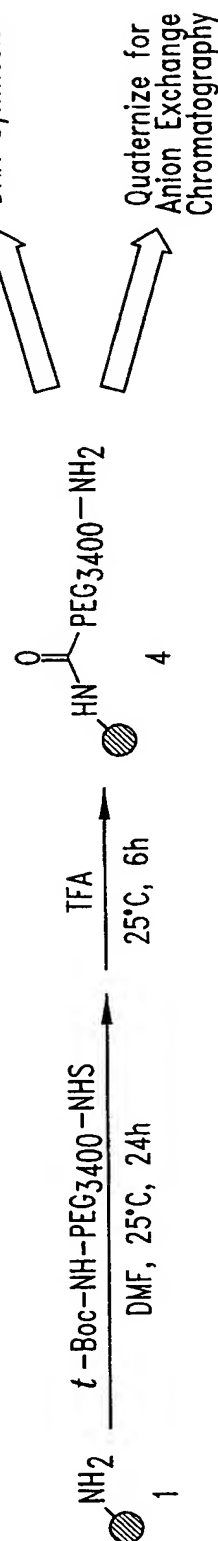


Fig. 6

Building Dendritic Molecules on AFR-Treated Materials and Their Modification for Use in DNA Synthesis and Anion Exchange Chromatography

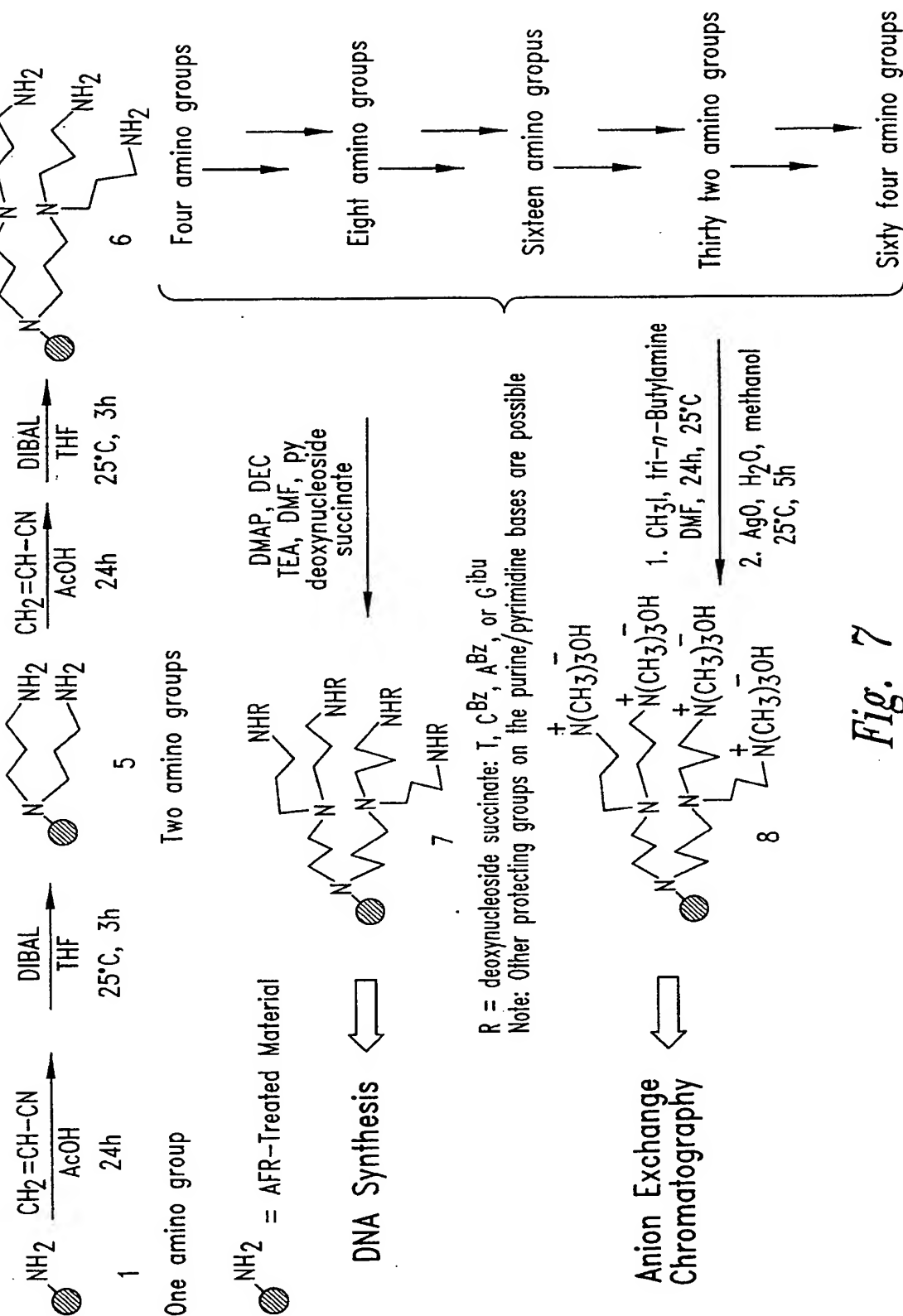
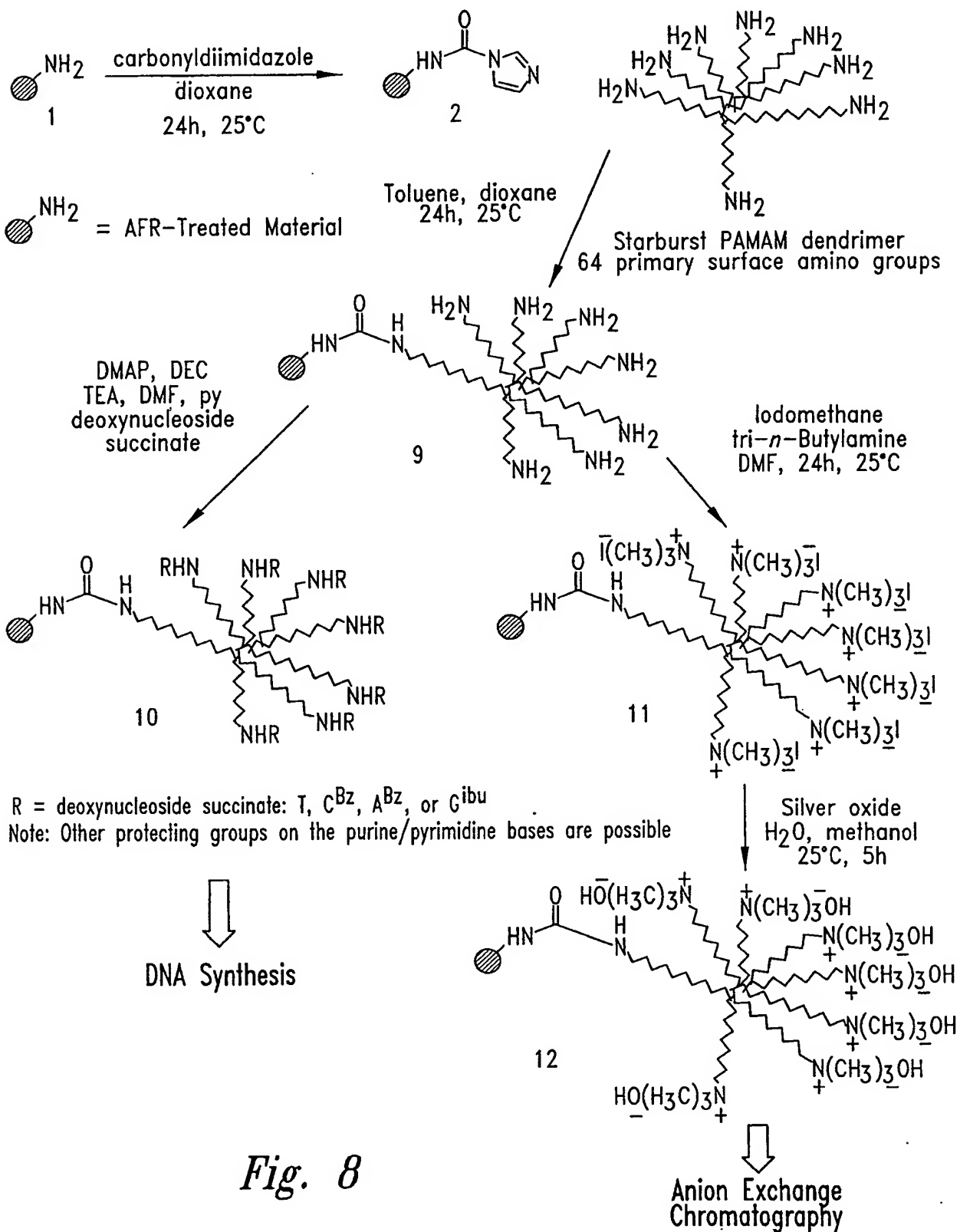


Fig. 7

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Attachment of Dendritic Molecules to AFR-Treated Materials and Their Modification for Use in DNA Synthesis and Ion Exchange Chromatography



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Attachment of StarPEG onto Amino FlowRad-Treated Materials

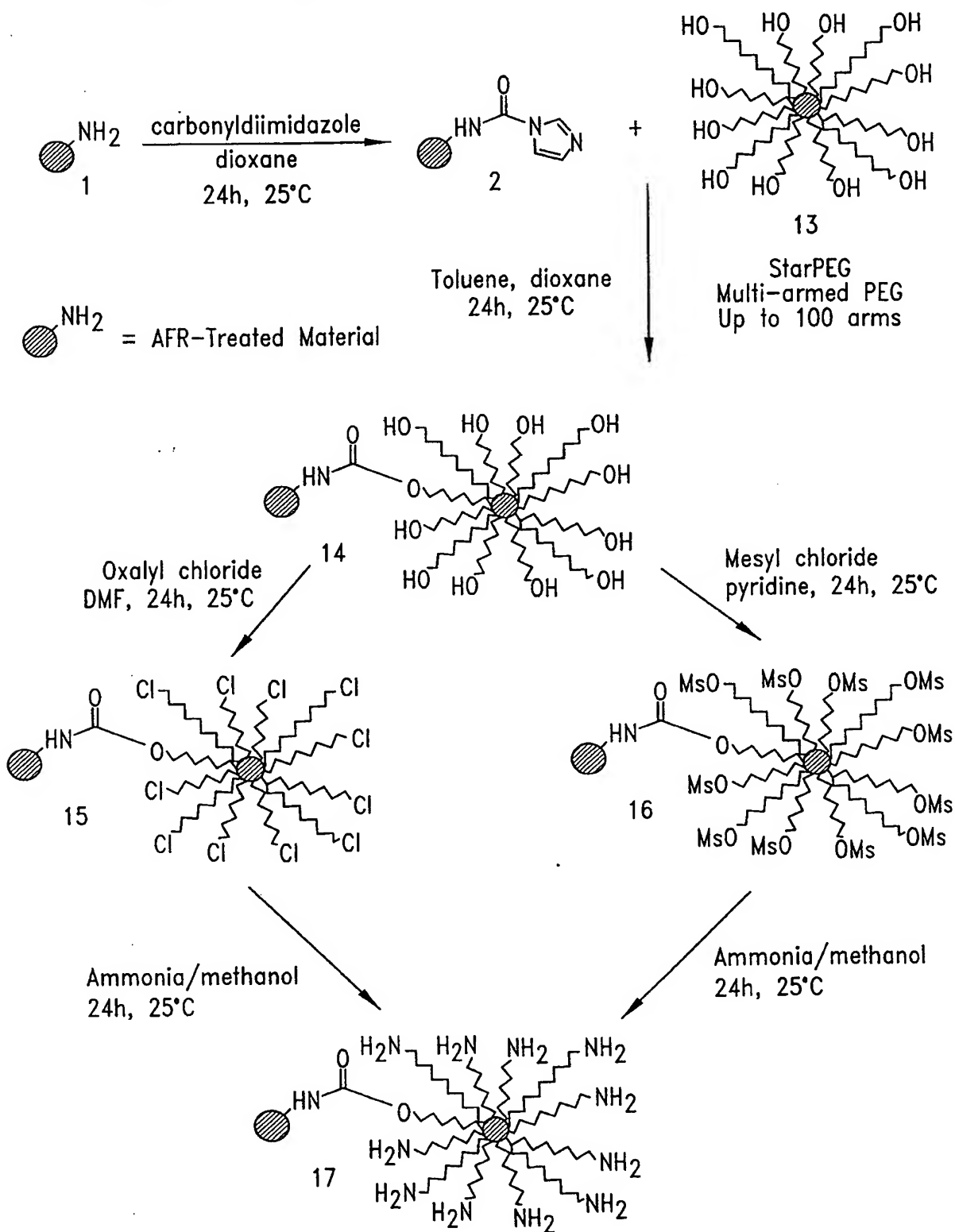


Fig. 9

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Chemical Modification of PEGylated Materials for DNA Synthesis and for Anion Exchange Chromatography

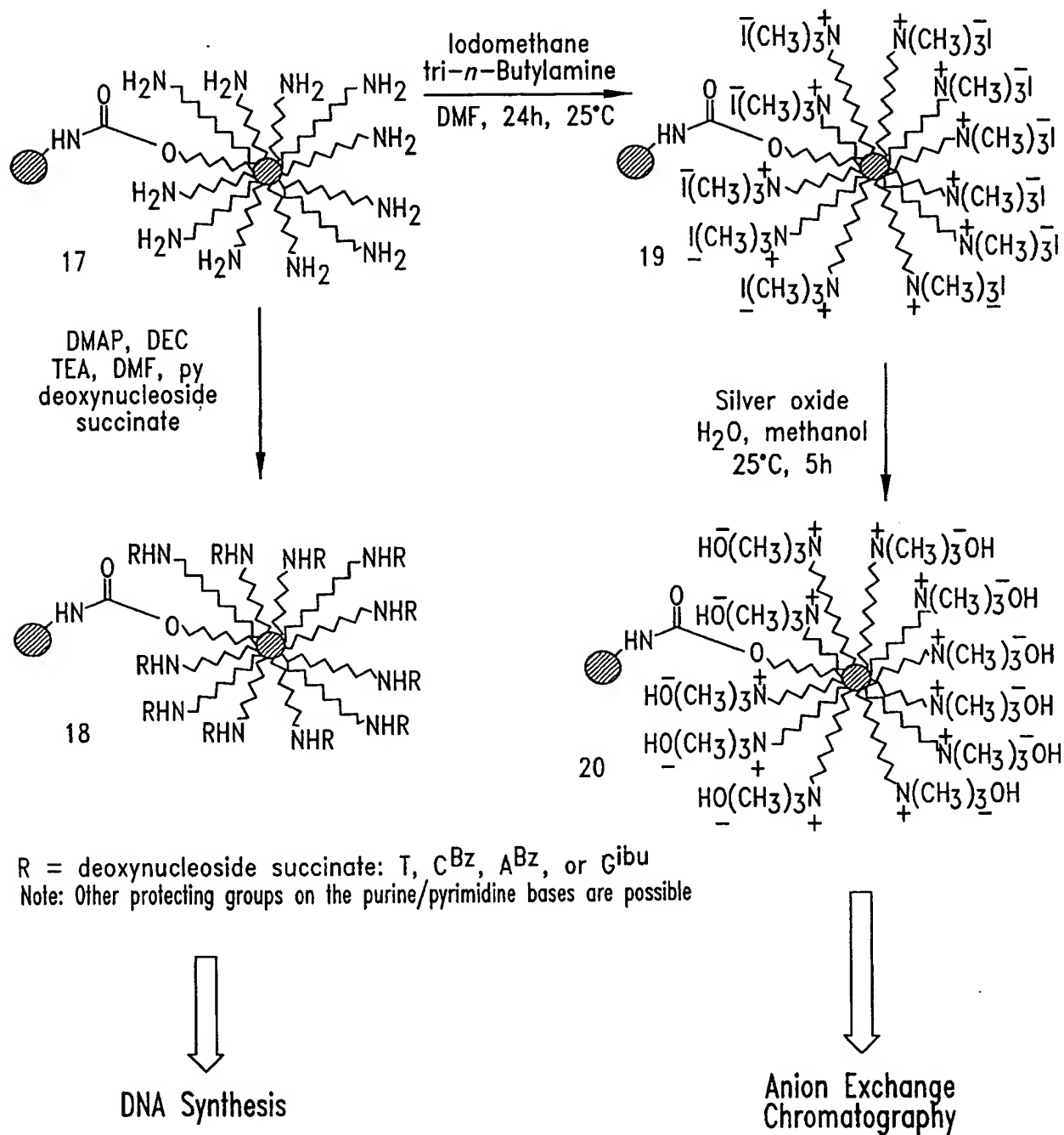


Fig. 10

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Acrylamide gel electrophoresis and densitometry analysis of
15-base long oligonucleotides prepared on Nanolith.

16-61 = example 27.9

16-62 = example 27.10

16-63 = example 27.11



Fig. 11A

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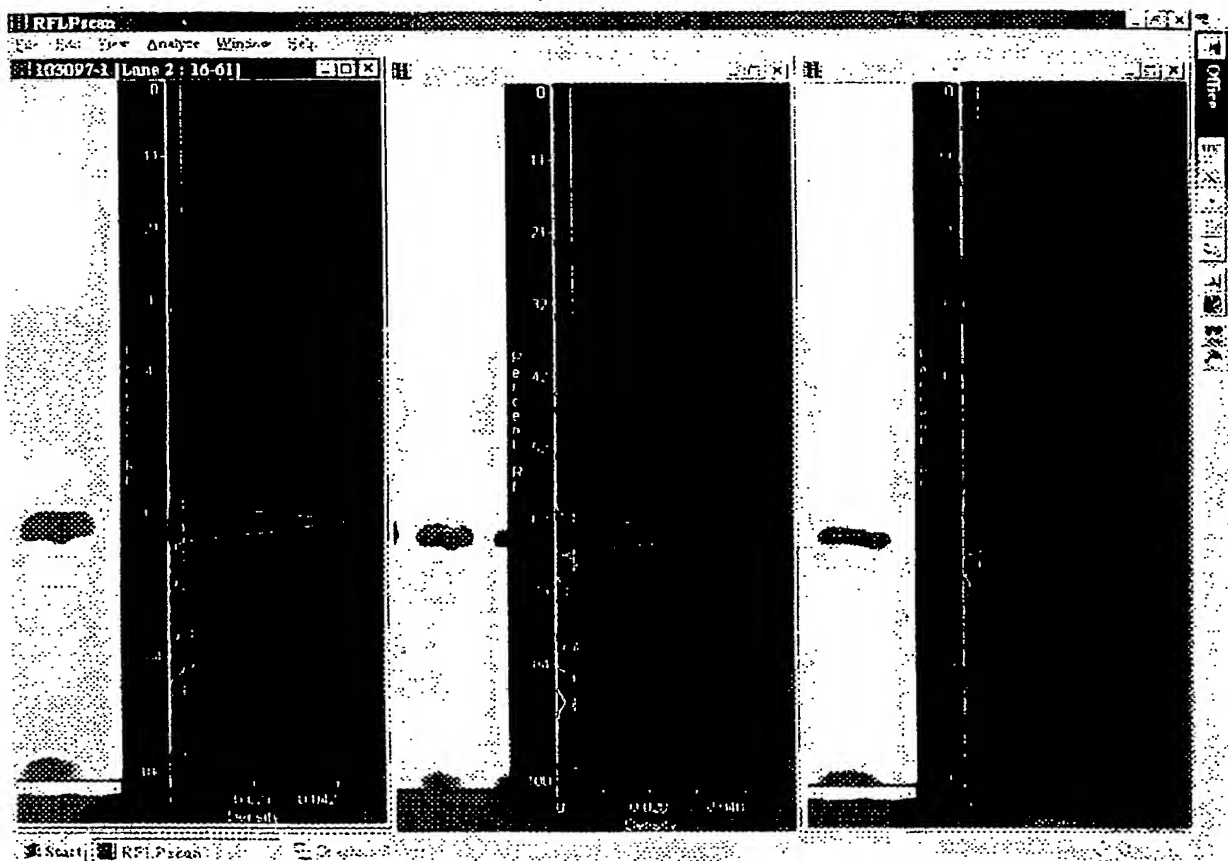


Fig. 11B

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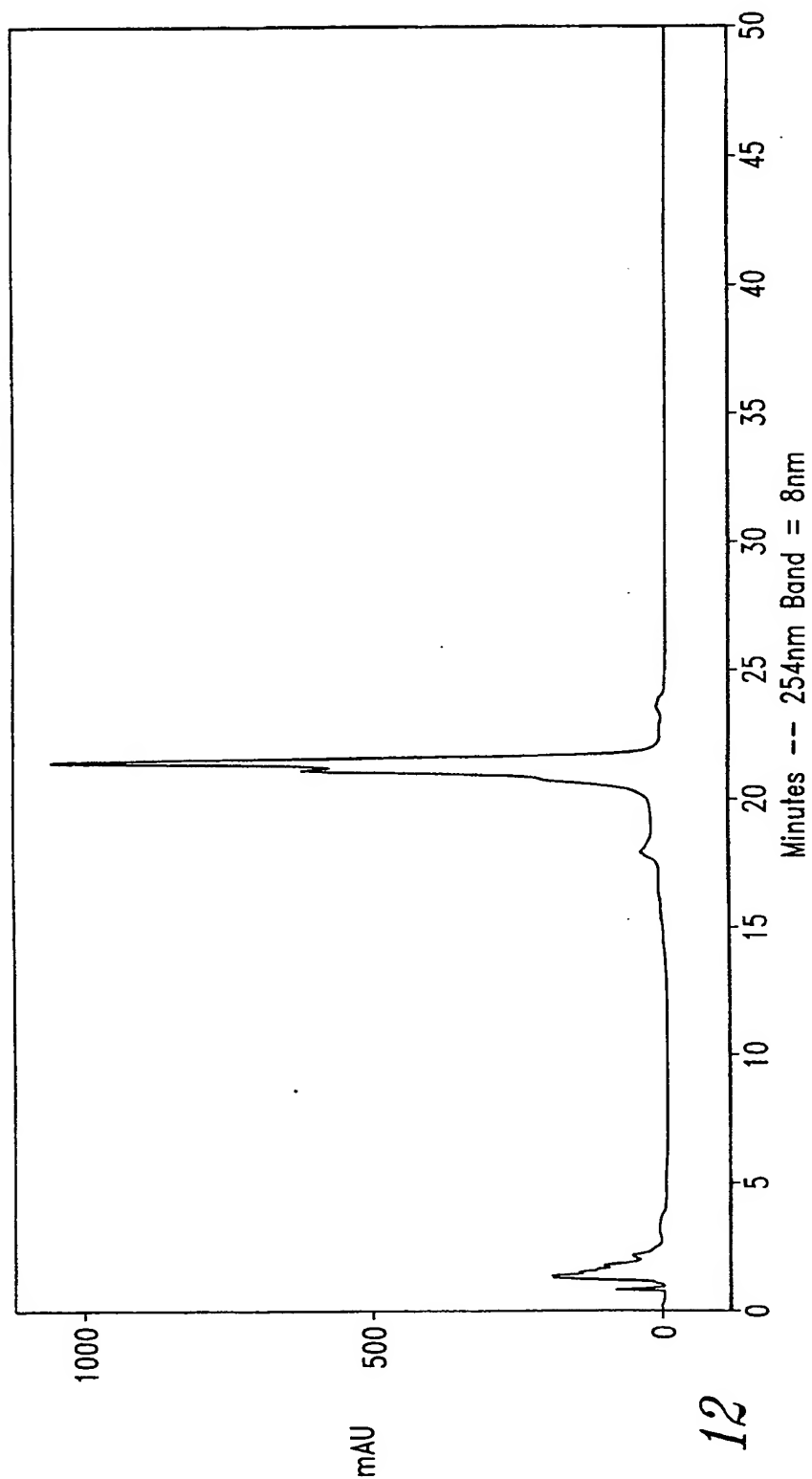
Band Report										
Sample name	Lane	% Rf	Ampl OD	IntOD OD	% IntOD	G.Loc pixel	G.Ampl OD	% Lane	IntOD	% Conc
16-61	2	63.2	0.0386229	0.284413	77.5	146.6	0.0390081		59.4	
16-61	2	68.4	0.00209785	0.0389963	10.6	150.8	0.00446821		8.1	
16-61	2	72.7	0.00159574	0.0095208	2.6	166.5	0.00224535		2.0	
16-61	2	79.7	0.0023489	0.0115739	3.2	184.7	0.00249564		2.4	
16-61	2	84.4	0.00366592	0.017025	4.6	195.5	0.00380297		3.6	
16-61	2	87.9	0.00145388	0.00566417	1.5	202.7	0.00152259		1.2	
16-62	3	63.6	0.0382843	0.262301	78.6	148.3	0.040032		62.5	
16-62	3	68.4	0.00465393	0.0220721	6.6	158.2	0.00471205		5.3	
16-62	3	72.3	0.00268006	0.0145853	4.4	167.1	0.00275279		3.5	
16-62	3	80.5	0.00172329	0.00874614	2.6	185.1	0.00199663		2.1	
16-62	3	85.3	0.00359297	0.0174549	5.2	196.2	0.00406211		4.2	
16-62	3	88.7	0.00159144	0.00838051	2.5	204.7	0.00158174		2.0	
16-63	4	64.8	0.0304329	0.164128	85.3	149.1	0.0310815		57.5	
16-63	4	68.7	0.00299835	0.0148009	7.7	158.5	0.00296471		5.2	
16-63	4	72.6	0.00145984	0.00651349	3.4	166.7	0.00126789		2.3	
16-63	4	86.1	0.00123858	0.00690802	3.6	196.7	0.00138547		2.4	

Fig. 11C

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Anion exchange chromatogram of a 15-base long oligonucleotides prepared on Nanolith (example 27.10)

Name	Time	Area	Area %
	17.94	1524073	3.93
	20.82	4987296	12.87
	21.14	10927850	28.20
	21.46	20725662	53.48
	22.85	178565	0.46
	23.59	284386	0.73
	23.79	126555	0.33
Totals:		38754388	100.00

*Fig. 12*

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Acrylamide gel electrophoresis of 15-base long
oligonucleotides prepared on various supports.

16-86 = Nanolith (example 27.10)

16-87 = TentaGel

16-88 = ArgoGel

16-89 = CPG (500 Å)

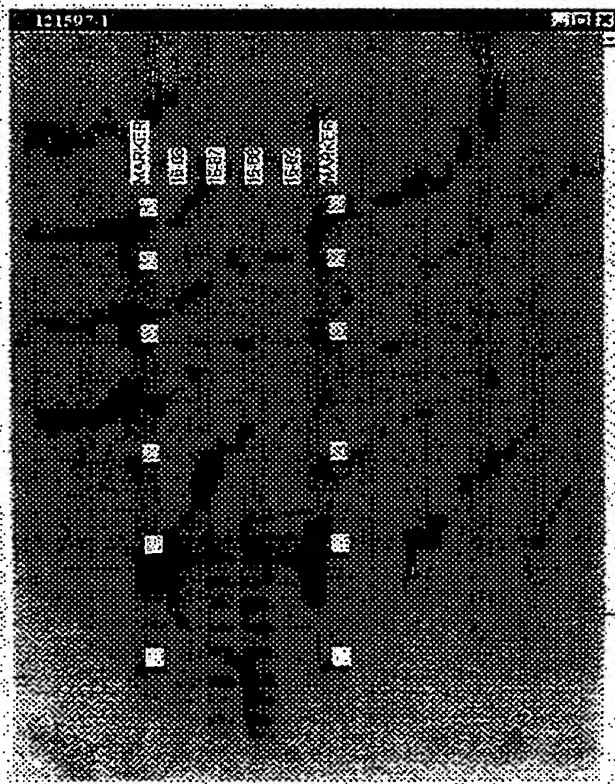


Fig. 13

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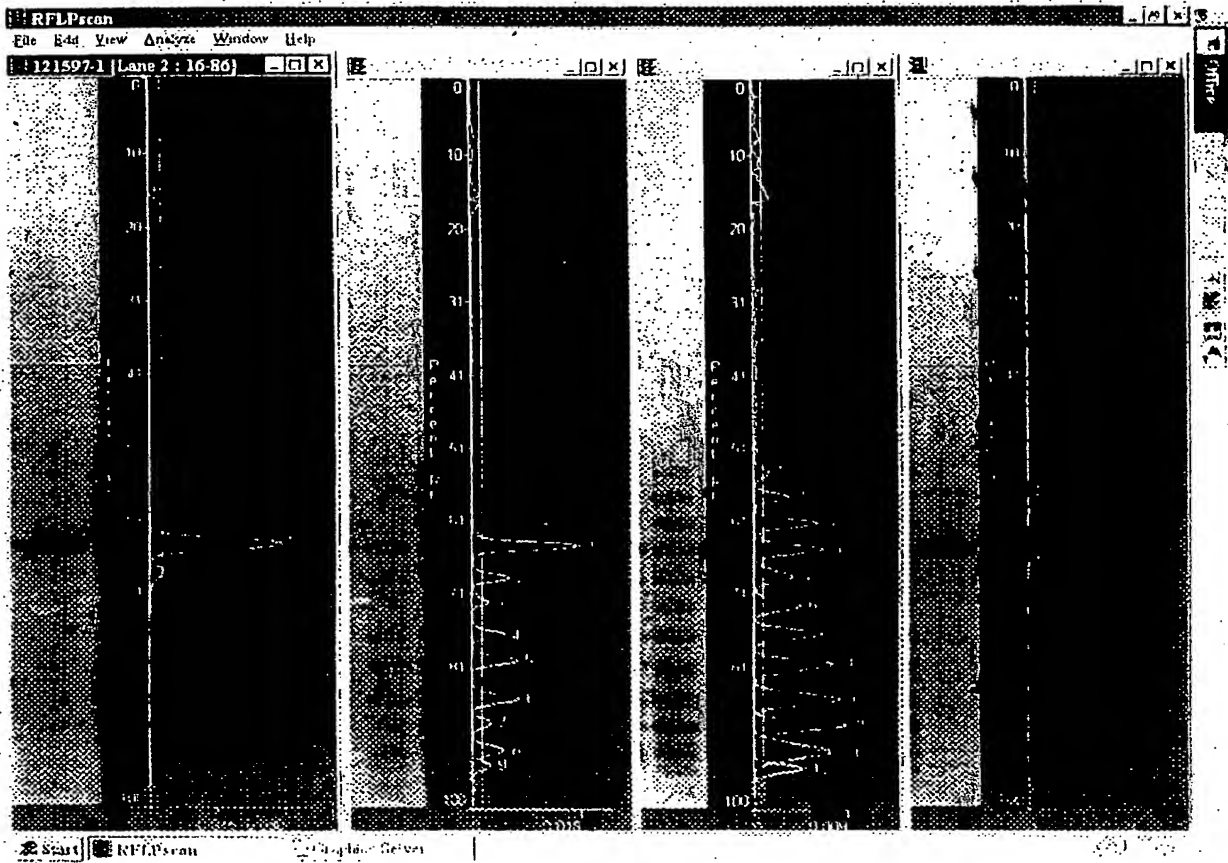


Fig. 1A

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Densitometry analysis of 15-base long oligonucleotides prepared on various supports.

Band Report										
Sample name	Lane	% Rf	Ampl OD	IntOD OD	% IntOD	G.Loc pixel	G.Ampl OD	% Lane	IntOD	% Conc
16-86	2	63.7	0.0282254	0.27122	88.8	328.3	0.0295241		55.2	
16-86	2	67.8	0.0030663	0.0341603	11.2	348.6	0.00297954		7.0	
16-87	3	64.1	0.0174859	0.113429	29.1	328.8	0.0179066		23.4	
16-87	3	68.4	0.00621605	0.0365922	9.4	351.3	0.00627112		7.6	
16-87	3	71.9	0.0023959	0.0149367	3.8	369.0	0.00259173		3.1	
16-87	3	76.4	0.00544143	0.0325319	8.3	391.9	0.00537269		6.7	
16-87	3	79.9	0.00756812	0.0502994	12.9	410.0	0.00764687		10.4	
16-87	3	85.2	0.00790954	0.0557576	14.3	437.2	0.00773784		11.5	
16-87	3	88.5	0.0033443	0.0236445	6.1	454.3	0.00326066		4.9	
16-87	3	92.4	0.00544691	0.0408663	10.5	473.8	0.00530929		8.4	
16-87	3	94.3	0.00294852	0.0216861	5.6	484.9	0.00298689		4.5	
16-88	4	53.5	0.00140715	0.0111821	1.9	276.1	0.00140422		1.7	
16-88	4	57.0	0.00328398	0.0221779	3.8	293.7	0.00336969		3.3	
16-88	4	60.9	0.0062151	0.040804	6.9	312.4	0.00604338		5.9	
16-88	4	64.4	0.00690675	0.0485358	8.4	331.2	0.00654584		7.2	
16-88	4	68.3	0.00386167	0.0350325	6.1	350.7	0.00359278		5.2	
16-88	4	72.2	0.0044775	0.0376084	6.5	371.1	0.00451964		5.6	
16-88	4	76.1	0.00649858	0.054616	9.4	391.6	0.00657863		8.1	
16-88	4	80.2	0.00791502	0.0677147	11.7	413.4	0.00745693		10.0	
16-88	4	85.2	0.00813246	0.0759789	13.1	437.8	0.00813338		11.3	
16-88	4	88.5	0.00880146	0.0697208	12.1	456.2	0.00875		10.3	
16-88	4	92.6	0.0078795	0.0863796	14.9	474.7	0.00730664		12.8	
16-88	4	94.7	0.00465751	0.0291627	5.0	487.1	0.00443505		4.3	
16-89	5	56.4	0.00568056	0.0278921	9.3	289.7	0.00574568		6.0	
16-89	5	64.0	0.0282316	0.271972	90.7	329.8	0.0294626		58.9	

Fig. 14B

16-86 = Nanolith
(example 27.10)

16-87 = TentGel

16-88 = ArgoGel

16-89 = CPG(500 Å)

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Trityl record (coupling efficiency) for a 75-base long oligonucleotide prepared on Nanolith
(example 27.10)

Final DMT: Removed

Sequence Name: F126

Sequence: (3') ACGATCGATCGATCATCGAGATCGCGATCTCGATATCGAGATCGCGATCTGATCTCG
58 ATATCGAGATCGCGATCT

Raw integrated values (read 3'-5' left to right):

7.10E5	7.98E5	8.86E5	8.04E5	8.25E5	7.79E5	7.22E5	7.73E5
8.93E5	9.04E5	7.88E5	9.70E5	1.03E6	1.01E6	1.01E6	1.04E6
1.04E6	8.69E5	1.01E6	8.44E5	9.57E5	1.03E6	9.84E5	8.70E5
9.97E5	8.77E5	9.40E5	9.66E5	9.53E5	9.57E5	9.08E5	7.96E5
8.66E5	8.91E5	9.15E5	8.99E5	8.83E5	7.66E5	8.65E5	7.75E5
8.38E5	8.82E5	8.75E5	7.44E5	8.46E5	7.19E5	8.32E5	8.49E5
8.32E5	8.54E5	6.59E5	7.87E5	7.95E5	8.09E5	8.42E5	7.73E5
6.79E5	7.47E5	7.82E5	7.94E5	7.91E5	8.12E5	6.90E5	7.60E5
6.64E5	7.86E5	7.78E5	7.58E5	6.52E5	7.72E5	6.78E5	7.64E5
7.62E5	7.56E5	7.71E5					

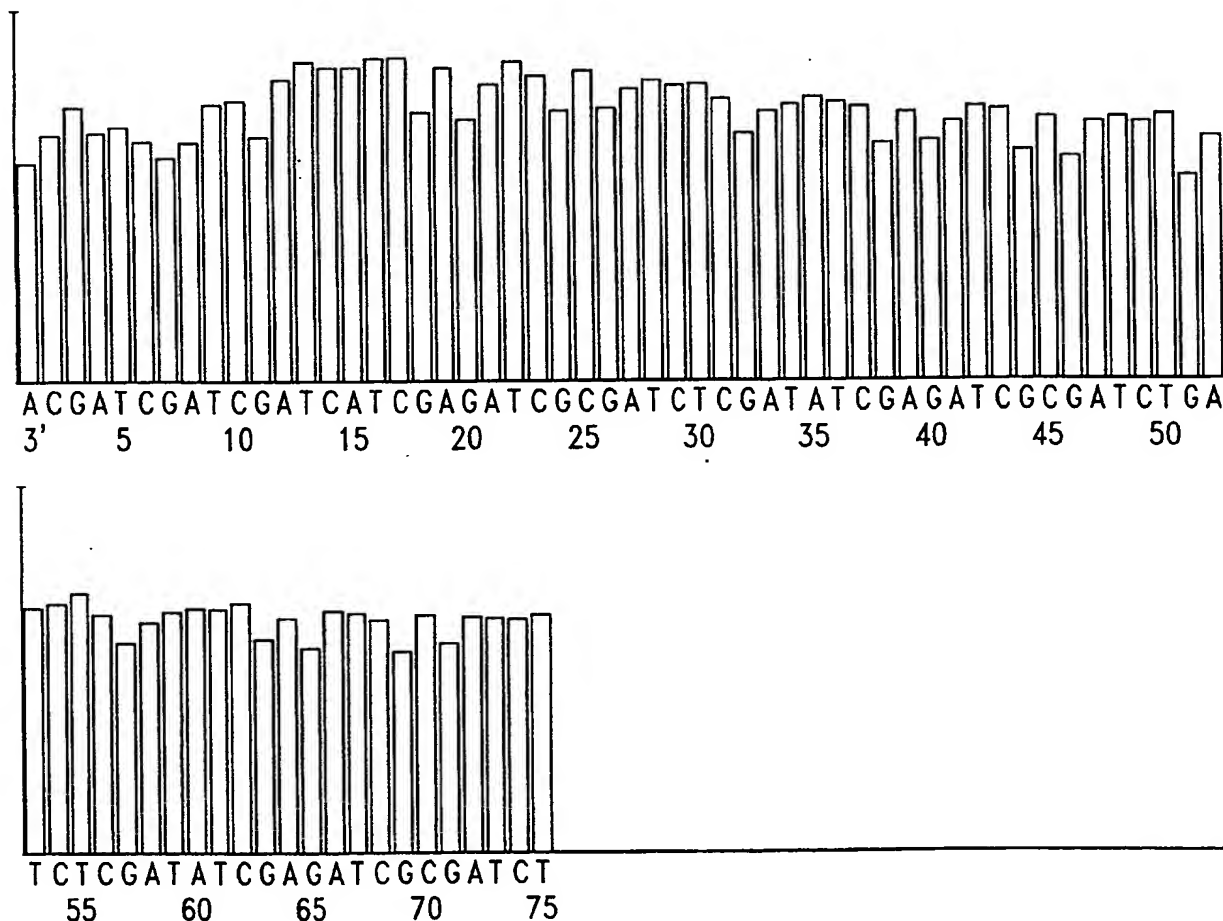


Fig. 15

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Trityl record (coupling efficiency) for a 200-base long oligonucleotide prepared on Nanolith
(example 27.10)

Final DMT: Removed

Sequence Name: F129

Sequence: (3') TCGATCGATCGATCATCGAGATCGCGATCTCGATATCGAGATCGCGATCTGATCTCG
58 ATATCGAGATCGCGATCTGATCTCGATATCGAGATCGCGATCTTCGATCGATCGATC
115 ATCGAGATCGCGATCTCGATATCGAGATCGCGATCTGATCTCGATATCGAGATCGCG
172 ATCTGATCTCGATATCGAATCGCGATCTA

Raw integrated values (read 3'-5' left to right):

9.32E5	9.35E5	7.17E5	9.77E5	8.53E5	9.04E5	6.83E5	8.53E5
9.29E5	8.34E5	8.01E5	9.21E5	8.27E5	8.00E5	8.06E5	8.35E5
8.94E5	7.96E5	8.13E5	7.13E5	8.33E5	8.53E5	8.47E5	7.44E5
8.66E5	7.93E5	8.07E5	8.37E5	8.25E5	8.44E5	7.95E5	7.37E5
8.33E5	8.26E5	8.43E5	8.46E5	7.86E5	7.59E5	7.92E5	7.50E5
8.05E5	8.10E5	7.96E5	7.44E5	7.88E5	7.16E5	8.22E5	8.07E5
7.70E5	8.01E5	7.65E5	7.81E5	7.87E5	7.87E5	8.13E5	8.19E5
7.05E5	7.84E5	8.10E5	7.43E5	8.31E5	7.84E5	7.12E5	7.66E5
7.29E5	7.40E5	7.67E5	7.77E5	6.92E5	7.25E5	7.05E5	7.61E5
7.71E5	7.62E5	7.63E5	6.60E5	7.77E5	7.68E5	7.39E5	7.49E5
7.69E5	6.87E5	7.37E5	7.65E5	7.54E5	7.70E5	7.11E5	7.10E5
7.59E5	6.95E5	7.19E5	7.45E5	7.61E5	6.56E5	7.53E5	6.31E5
7.06E5	7.28E5	7.54E5	7.48E5	7.87E5	7.53E5	6.98E5	7.42E5
7.65E5	7.25E5	6.84E5	7.44E5	7.56E5	7.43E5	6.90E5	7.50E5
7.47E5	7.27E5	7.33E5	7.68E5	7.45E5	6.70E5	7.31E5	6.35E5
7.15E5	7.50E5	7.33E5	6.65E5	7.20E5	6.44E5	6.86E5	7.60E5
7.64E5	7.36E5	7.15E5	6.48E5	7.10E5	7.25E5	7.28E5	7.28E5
6.78E5	5.91E5	6.37E5	6.31E5	6.35E5	7.16E5	6.60E5	6.14E5
6.87E5	5.84E5	7.00E5	7.00E5	6.95E5	7.10E5	6.23E5	6.73E5
7.15E5	7.16E5	7.12E5	7.14E5	6.12E5	6.80E5	7.18E5	7.32E5
6.53E5	7.22E5	6.06E5	7.18E5	5.83E5	6.80E5	7.26E5	7.30E5
6.15E5	6.76E5	5.87E5	7.09E5	7.04E5	6.89E5	7.24E5	6.21E5
6.98E5	7.11E5	7.17E5	7.35E5	7.63E5	6.02E5	7.24E5	7.39E5
6.82E5	7.40E5	7.50E5	6.44E5	7.03E5	6.83E5	7.04E5	7.81E5
6.30E5	7.32E5	5.95E5	6.86E5	8.03E5	6.97E5	7.98E5	8.95E5

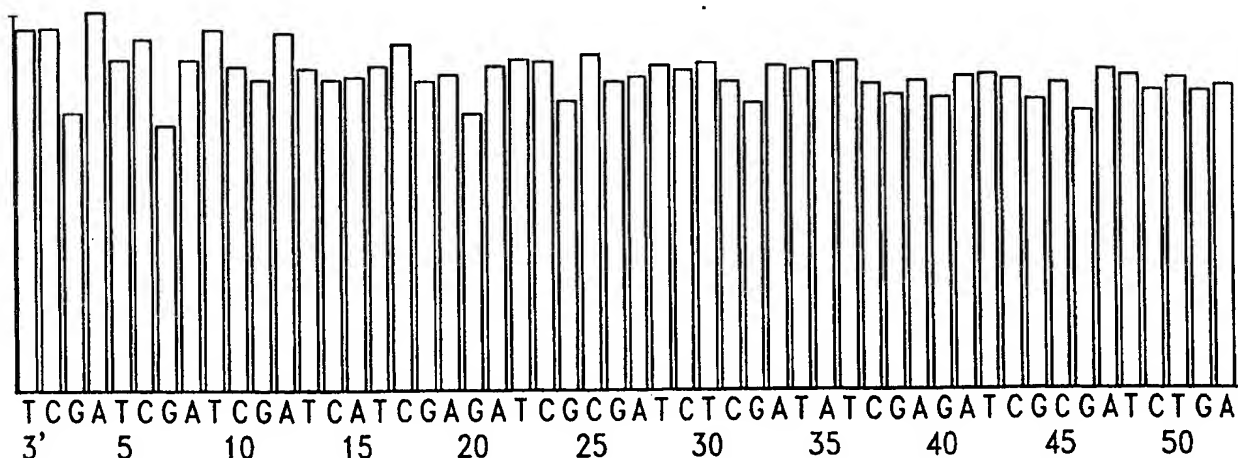
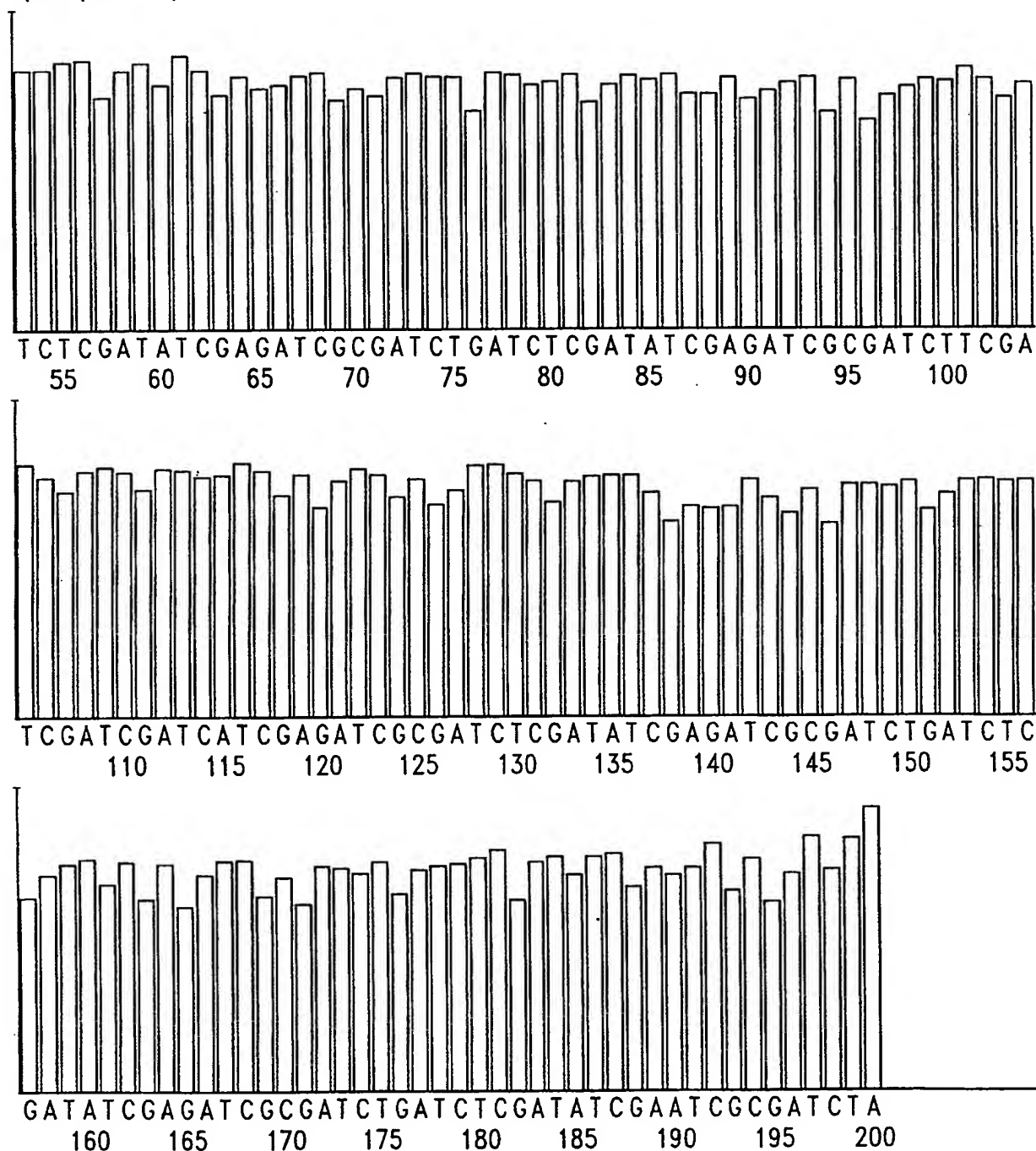


Fig. 16A

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Trityl record (coupling efficiency) for a 200-base long oligonucleotide prepared on Nanolith
(example 27.10)

*Fig. 16B*

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Trityl record (coupling efficiency) for a 200-base long oligonucleotide prepared on 1000Å CPG

Final DMT: Removed

Sequence Name: F129

Sequence: (3') TCGATCGATCGATCATCGAGATCGCGATCTCGATATCGAGATCGCGATCTGATCTCG
 58 ATATCGAGATCGCGATCTGATCTCGATATCGAGATCGCGATCTTCGATCGATCGATC
 115 ATCGAGATCGCGATCTCGATATCGAGATCGCGATCTGATCTCGATATCGAGATCGCG
 172 ATCTGATCTCGATATCGAATCGCGATCTA

Raw integrated values (read 3'-5' left to right):

3.58E5	9.34E5	7.75E5	9.08E5	9.01E5	8.52E5	7.06E5	7.57E5
7.65E5	7.38E5	6.53E5	6.91E5	7.53E5	7.07E5	6.86E5	6.92E5
6.66E5	5.65E5	6.47E5	5.71E5	6.31E5	6.77E5	6.68E5	5.81E5
6.78E5	6.21E5	6.74E5	6.85E5	6.57E5	6.65E5	6.47E5	5.88E5
6.22E5	6.48E5	6.01E5	6.57E5	5.85E5	5.41E5	5.83E5	5.54E5
5.70E5	5.97E5	5.80E5	5.65E5	6.04E5	5.35E5	5.67E5	6.35E5
6.16E5	5.98E5	5.52E5	5.62E5	5.83E5	5.68E5	6.11E5	5.67E5
5.47E5	5.46E5	5.95E5	5.42E5	5.88E5	6.21E5	5.13E5	5.89E5
5.31E5	5.61E5	6.12E5	6.13E5	5.71E5	6.39E5	5.48E5	5.96E5
6.62E5	5.90E5	6.18E5	5.69E5	5.93E5	6.45E5	6.35E5	6.11E5
6.35E5	5.95E5	6.31E5	6.86E5	6.71E5	7.07E5	6.78E5	6.00E5
6.67E5	6.07E5	6.92E5	7.36E5	7.28E5	6.17E5	7.11E5	6.36E5
7.04E5	7.30E5	7.11E5	7.40E5	7.80E5	7.41E5	6.79E5	7.19E5
7.38E5	7.23E5	7.37E5	7.35E5	6.88E5	6.72E5	6.98E5	6.78E5
6.77E5	6.36E5	6.31E5	6.02E5	5.76E5	6.12E5	5.39E5	5.66E5
5.23E5	4.89E5	4.95E5	5.16E5	4.50E5	4.90E5	4.53E5	4.65E5
4.23E5	4.09E5	4.20E5	4.04E5	4.01E5	4.10E5	3.61E5	3.63E5
3.88E5	3.52E5	3.65E5	3.32E5	3.37E5	3.15E5	3.45E5	3.19E5
3.43E5	2.85E5	2.88E5	2.67E5	2.97E5	3.07E5	2.79E5	2.60E5
2.57E5	2.56E5	3.07E5	2.59E5	2.62E5	2.75E5	2.51E5	2.46E5
2.94E5	2.85E5	2.38E5	2.54E5	2.56E5	2.50E5	2.55E5	2.69E5
2.12E5	2.41E5	2.58E5	2.41E5	2.48E5	2.42E5	2.36E5	2.20E5
2.25E5	2.12E5	2.24E5	2.29E5	2.52E5	2.07E5	2.03E5	2.39E5
2.11E5	2.06E5	1.99E5	2.17E5	1.99E5	2.04E5	2.08E5	2.05E5
1.81E5	1.83E5	1.93E5	1.73E5	1.71E5	1.77E5	1.72E5	1.64E5

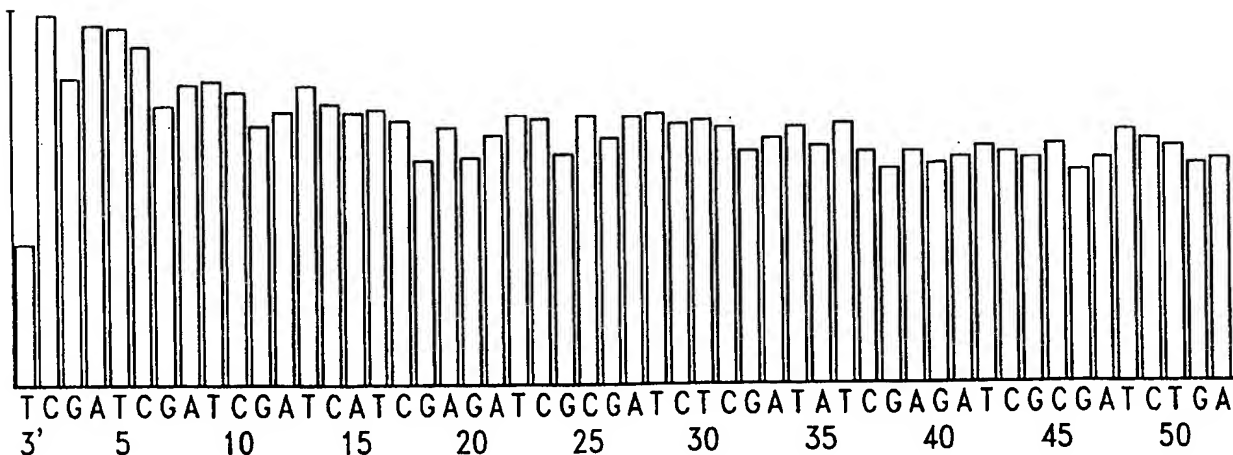
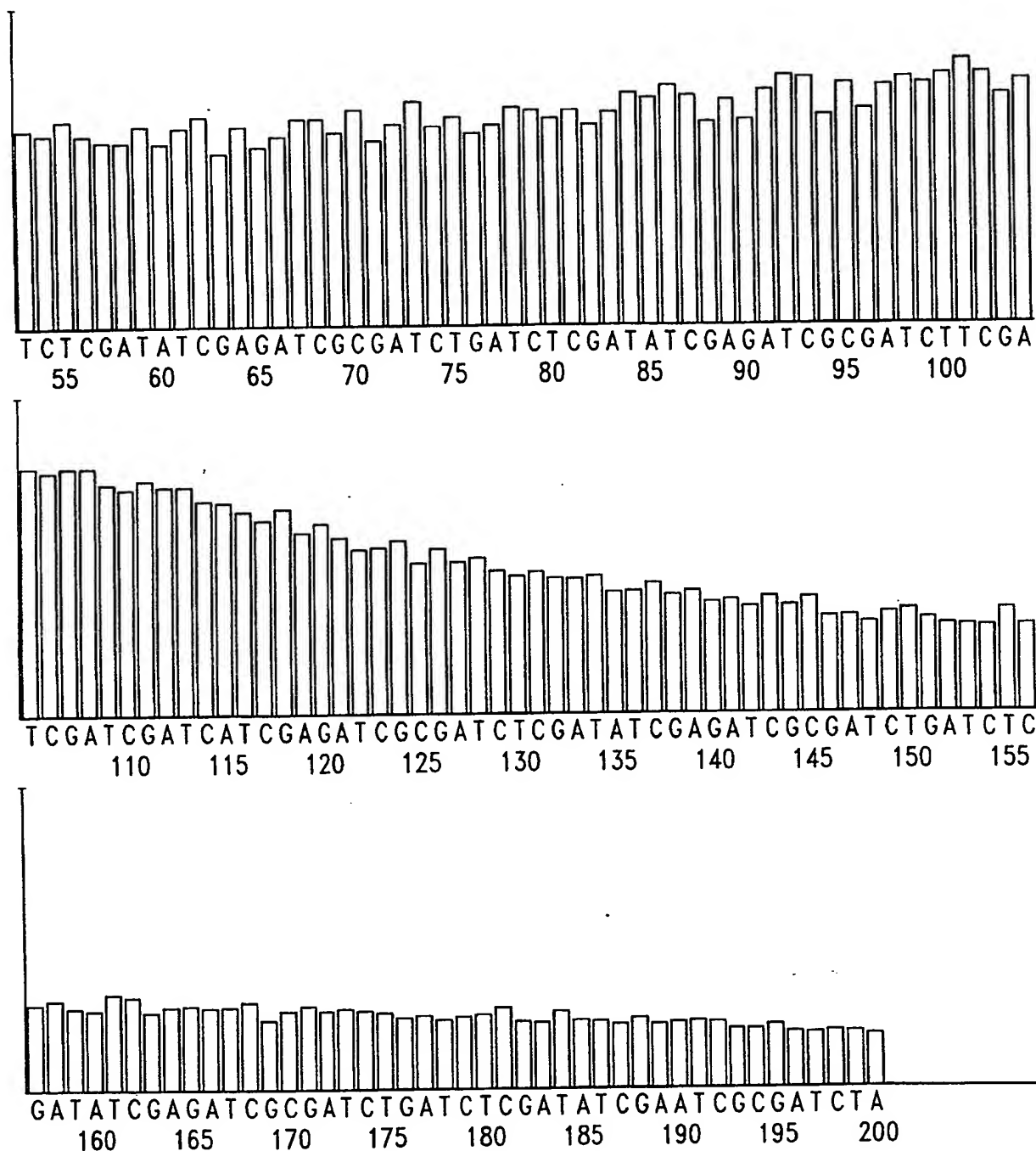


Fig. 17A

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Trityl record (coupling efficiency) for a 200-base long oligonucleotide prepared on 1000Å CPG

*Fig. 17B*

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/01278

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C08J9/36 C08J7/12 B01D67/00 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C08J B01D G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 010, no. 090 (C-337), 8 April 1986 & JP 60 221441 A (BRIDGESTONE KK), 6 November 1985, see abstract ---	1-59
A	US 4 597 828 A (TADROS JEHANE) 1 July 1986 see column 3, line 26-38 see claims 1,8,9 ---	1-59
A	PATENT ABSTRACTS OF JAPAN vol. 014, no. 505 (C-0775), 5 November 1990 & JP 02 208333 A (TONEN CORP), 17 August 1990, see abstract ---	1-59
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

8 June 1998

Date of mailing of the international search report

23/06/1998

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INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/US 98/01278

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 96 23834 A (SCHNEIDER USA INC) 8 August 1996 see claims</p> <p>-----</p>	1-59

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01278

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4597828 A	01-07-1986	NONE	
WO 9623834 A	08-08-1996	CA 2211160 A	08-08-1996
		EP 0807140 A	19-11-1997
		JP 10502857 T	17-03-1998

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